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Research review paper

Reactor design for minimizing product inhibition during enzymatic lignocellulose hydrolysis
II. Quantification of inhibition and suitability of membrane reactors

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ABSTRACT

Product inhibition of cellulolytic enzymes affects the efficiency of the biocatalytic conversion of lignocellulosic biomass to ethanol and other valuable products. New strategies that focus on reactor designs encompassing product removal, notably glucose removal, during enzymatic cellulose conversion are required for alleviation of glucose product inhibition. Supported by numerous calculations this review assesses the quantitative aspects of glucose product inhibition on enzyme-catalyzed cellulose degradation rates. The significance of glucose product inhibition on dimensioning of different ideal reactor types, i.e. batch, continuous stirred, and plug-flow, is illustrated quantitatively by modeling different extents of cellulose conversion at different reaction conditions. The main operational challenges of membrane reactors for lignocellulose conversion are highlighted. Key membrane reactor features, including system set-up, dilution rate, glucose output profile, and the problem of cellobiose are examined to illustrate the quantitative significance of the glucose product inhibition and the total glucose concentration on the cellulolytic conversion rate. Comprehensive overviews of the available literature data for glucose removal by membranes and for cellulose enzyme stability in membrane reactors are given. The treatise clearly shows that membrane reactors allowing continuous, complete, glucose removal during enzymatic cellulose hydrolysis, can provide for both higher cellulose hydrolysis rates and higher enzyme usage efficiency ($kg_{product}/kg_{enzyme}$). Current membrane reactor designs are however not feasible for large scale operations. The report emphasizes that the industrial realization of cellulosic ethanol requires more focus on the operational feasibility within the different hydrolysis reactor designs, notably for membrane reactors, to achieve efficient enzyme-catalyzed cellulose degradation.

Contents

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1. Introduction

Product inhibition of cellulases by cellobiose and glucose has long been known to significantly retard the rates of enzyme-catalyzed cellulose hydrolysis (Gan et al., 2003; Gusakov et al., 1987). This inhibition constitutes a main obstacle for achieving efficient enzymatic degradation of cellulose and high glucose yields in current lignocellulose-to-ethanol processing schemes (Andrić et al., 2010a; Bélafi-Bakó et al., 2006; Xiao et al., 2004). The product inhibition of cellulolytic enzymes also affects the efficiency of other processes involving conversion of lignocellulosic biomass to valuable products. Alleviation of this product inhibition, notably the inhibition by the hydrolysis end-product glucose, is therefore a key prerequisite for achieving cost-efficient conversion of lignocellulosic biomass to biofuels — notably bioethanol and biobutanol — and other valuable products such as platform biochemicals. A number of glucose tolerant fungal β-glucosidases, produced by various *Aspergillus* spp. and e.g. *Humicola insolens*, have been identified relatively recently (Decker et al., 2001; Sonia et al., 2008), but the prospects of developing and using glucose tolerant enzymes seem to receive surprisingly limited attention in the commercial enzyme development for biomass utilization. Rather, the industrial focus has mainly been on reducing the enzyme costs by improving the efficiency of known enzymes, identifying new, more active enzymes, creating optimal enzyme mixtures for selected pre-treated substrates, and on minimizing the enzyme production costs (Merino and Cherry, 2007; Rosgaard et al., 2007b). A careful analysis of the mechanisms and kinetics of the product inhibition induced by glucose and cellobiose on microbial cellulases and β-glucosidase has substantiated that reactor designs which involve continuous or semi-continuous product removal — notably glucose removal — must be at the core of future-directed design strategies for lignocellulose-to-ethanol processes (Andrić et al., 2010b).

Simultaneous saccharifaction and fermentation (SSF), with or without separate fermentation of pentose monosaccharides, is considered a main technology scenario in current biomass-to-ethanol processes (Hahn-Hägerdal et al., 2006; Lynd et al., 2008). Although alleviation of product inhibition is a rationale for SSF, it seems to have been overlooked that the efficiency of this technology is restricted by the inhibition that the ethanol exerts on the cellulolytic enzymes (Bezerra and Dias, 2005). Hence, a certain degree of separate enzymatic hydrolysis of the cellulosic biomass appears to be the most feasible approach for accomplishing the enzymatic degradation of cellulose to glucose in future large scale cellulose-to-ethanol processes and in other lignocellulosic biomass upgrading processes as well.

The purpose of this review is to examine the quantitative effects of product removal on lignocellulose hydrolysis efficiency, i.e. the influence of glucose removal on the rates and extents of conversion in enzymatic cellulose hydrolysis, and to discuss the key reactor design issues, operational features, and the overall advantages and disadvantages of membrane reactors for glucose product removal during cellulolytic enzyme hydrolysis. By highlighting the immense potential as well as the challenges that lie ahead in the development of reactor systems that reduce the product inhibition of cellulases, our objective is to provide an improved knowledge-base for rationally designing reactor systems for efficient enzymatic cellulose hydrolysis. The present review is tightly connected to another report which examines the reaction mechanisms and product inhibition kinetics on enzymatic cellulose hydrolysis in relation to the particular complexities of enzyme-catalyzed cellulose hydrolysis (Andrić et al., 2010b).

1.1. Influence of product inhibition on enzyme-catalyzed rates

The effects of inhibitors — especially their influence on the initial reaction rate — have been extensively studied in classical enzyme kinetics and enzymology. The evaluation of enzyme inhibition has for example for a long time been one of the major methods used in pharmacological research to analyze and quantify the action of drugs and in drugs development (Levenspiel, 1993). It is of course also well known that product inhibition can hinder the obtainment of high yields and high converison rates in industrial enzyme technology (Riebel and Bommarius, 2004; Frieden and Walter, 1963; Fullbrook, 1996). However, apart from a few important cases (e.g. lactose hydrolysis), the negative effects of product inhibition has surprisingly rarely led to drastic changes in processing regimes and reactor design in large scale industrial enzyme reactions. If product inhibition had been more in focus it is our presumption that significantly fewer simple batch reactors and batch reactions would be in place in industrial enzyme technology.