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Research paper

Investigation of adsorption kinetics and isotherm of cellulase and β-glucosidase on lignocellulosic substrates

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ABSTRACT

Clear understanding of enzyme adsorption during enzymatic hydrolysis of lignocellulosic biomass is essential to enhance the cost-efficiency of hydrolysis. However, conclusions from literature often contradicted each other because enzyme adsorption is enzyme, biomass/pretreatment and experimental condition specific, which makes descriptions and modeling of enzyme-substrate interaction difficult and inconsistent from case to case. This study investigated adsorption kinetics and isotherm under actual hydrolysis conditions with commercial cellulase and β-glucosidase on Avicel, dilute acid pretreated Creeping Wild Ryegrass (pCWR) and lignin residue of pCWR after enzymatic hydrolysis. It was found that β-glucosidase has little affinity to Avicel, but significant affinity to dilute acid pCWR and lignin with maximum adsorption capacity (E_{max}) of 161.57 and 173.50 mg protein/g-substrate, respectively. During hydrolysis, adsorption of cellulase on Avicel was productive and reversible (E_{max} = 22.86 mg protein/g-substrate); however, nonproductive and irreversible adsorption of cellulase on pCWR (E_{max} = 42.55 mg protein/g-substrate) and lignin (E_{max} = 86.07 mg protein/g-substrate) became significant and resulted in cellulase deactivation. Lignin is a key issue causing high cost of enzymatic hydrolysis of lignocellulosic biomass. The nonionic surfactant, Tween 20 was found to significantly overcome nonproductive adsorption of cellulase and β-glucosidase on lignin by reducing the adsorption affinity. All adsorption data including with and without Tween 20 were fit well to Langmuir isotherm. The results from this research will provide useful data for model development of enzymatic hydrolysis.

1. Introduction

Lignocellulosic biomass is an excellent renewable feedstock for biofuel and chemical production via sugar platform. Sugar production from lignocellulosic biomass on a commercial scale is, however, still hindered by technical and economic obstacles. Enzymatic cellulose hydrolysis for sugar production offers advantages over chemical conversion routes such as milder conditions, higher yields and minimal byproducts generation. While a lot of research has been done on enzymatic hydrolysis, the high cost rendered cellulosic ethanol economically unfit primarily because enzyme is costly along with high enzyme dosages required to achieve desired sugar yield. Cost-efficient pretreatment technologies and enzymatic hydrolysis (such as using better enzyme and process optimization) are the most commonly addressed approaches to make biomass amenable to enzyme attack. Hence, obtaining better understanding of interaction between enzyme and biomass substrate is important to enhance the cost-efficiency of enzymatic hydrolysis, which is a key factor to achieve economically feasible cellulosic ethanol. Enzymatic hydrolysis of lignocellulosic biomass is a heterogeneous biocatalytic process in which enzyme adsorption on substrates and the formation of enzyme-substrate complexes are prerequisites for cellulose hydrolysis, and such complexes are a central feature of most conceptual and quantitative models of enzymatic hydrolysis. As a result, it is essential to accurately describe enzyme adsorption behavior.

Although adsorption of commercial enzyme preparations (e.g. cellulase and β-glucosidase) and purified monocomponent enzymes (e.g. TrCel 5A and TrCel 7A) has been studied on various lignocellulosic substrates for decades, no universal conclusion has been drawn on the mechanism of enzyme actions. In some cases, the reported results were contradictory. The main reasons could be

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ascribed to the different enzyme/substrate/testing conditions used in different studies. It has been shown that the initial hydrolysis rate is proportional to the amount of cellulase initially adsorbed [1–3]. However, the rapid decrease of the hydrolysis rate indicated a rather low conversion of the substrate even though the amount of adsorbed enzyme had little change during hydrolysis. As a result, the relationship between hydrolysis rate and enzyme adsorption is still unclear. In addition, most of the published work showed that the adsorption equilibrium occurs within 10–20 min [4,5]. However, longer time ranging from 60 min to 8 h was also reported [6,7]. Most adsorption isotherms of cellulase on biomass were determined at low temperature (4–10 °C) to avoid the influence of hydrolysis reaction. However, the adsorption is strongly affected by temperature and the practical hydrolysis occurs between 40 °C and 50 °C [8–10]. Thus, the adsorption isotherms should be investigated at this temperature if the results are to be used for model development.

During enzymatic hydrolysis of lignocellulosic biomass, enzymes actually adsorb to both cellulose and lignin [11]. Productive binding of enzymes to cellulose results in sugar production, which is desired. Meanwhile, non-productive binding also occurs, i.e., enzymes either remain trapped in the dead ends of the substrate structure, or bind non-productively to cellulose [12] or to lignin [13]. So far, it has not been possible to differentiate between two groups of enzymes adsorbed to cellulose and lignin or between these two types of binding on complex substrates containing both cellulose and lignin. The non-productive cellulase adsorption to lignin is irreversible, consequently resulting in reduced hydrolysis rate/yield and enzyme recycle [14–17]. This should be considered in adsorption isotherm for modeling enzymatic hydrolysis. It has been reported that the adsorption of enzymes is influenced by the nature of lignin preparations [13,18,19]. Hence, lignin residue prepared by a complete enzymatic hydrolysis would be better than other lignin preparations such as alkali lignin for enzyme adsorption experiment. To prevent non-productive binding of cellulases to lignin, surfactants and non-catalytic protein (e.g. bovine serum albumin) can be added to enzymatic hydrolysis to reduce enzyme loading along with enhanced sugar yield [11,20]. Of all, non-ionic surfactants such as Tween 20 have proved to be the most effective and a promising technology to lower the cost of enzymatic hydrolysis. As a result, it is important to study the effect of surfactants on enzyme adsorption kinetics and isotherm on lignocellulosic substrates if the surfactants will be used.

Besides cellulase, β-glucosidase is another critical enzyme involved in biomass hydrolysis for hydrolyzing cellobiose into glucose. Since cellobiose is soluble and β-glucosidase hydrolysis is homogeneous catalysis process, β-glucosidase does not adsorb on solid substrate in theory. However, Yang and Wyman (2006) reported that β-glucosidase was adsorbed on dilute acid pretreated corn stover, particularly lignin, and lost activity [17]. Only a few studies have been done on β-glucosidase adsorption characteristics and their conclusions contradicted each other. Haven and Jorgensen (2013) reported that β-glucosidase (novozyme 188) from *Aspergillus niger* did not adsorb on lignin or biomass, while Cellic CTec 2 β-glucosidase adsorbed significantly [21]. The major reason was the differences of enzyme preparation, biomass and pretreatment. Therefore, adsorption studies for β-glucosidase should also be based on specific combinations of enzyme, biomass and pretreatment.

Peitersen et al. (1977) was the first to suggest to use Langmuir isotherm to quantify the cellulase adsorption isotherm [22]. After that, Langmuir isotherm has been extensively used since it provides a good fit to the adsorption data in most cases, and represents a simple mechanistic model that can be employed to compare the kinetic properties of various enzyme-cellulose systems [23]. However, it should be noted that the underlying assumptions for the Langmuir isotherm such as uniform binding sites and no interaction between adsorbed molecules are not applicable to cellulase adsorption on lignocellulosic substrate [6]. For the purpose of model development, the Langmuir isotherm is still a simple and useful tool to reflect the interaction between enzymes and substrates.

In this paper, the adsorption of commercial cellulase (Celluclast 1.5 L) and β-glucosidase (novozyme 188) on three structurally different substrates, including microcrystalline cellulose (Avicel), dilute acid pretreated Creeping Wild Ryegrass (pCWR) and lignin residue after enzymatic hydrolysis of pCWR, was studied with and without the presence of Tween 20. Our comprehensive research reflected all six possible enzyme-substrate adsorption scenarios occurring within enzymatic hydrolysis of pretreated lignocellulosic biomass. Unlike previous research in the literature, we used the same sourced pretreated biomass and lignin residue to make the adsorption study closer to practical conditions and result comparisons more consistent. The results from this research will provide useful data for model development of enzymatic hydrolysis. The objectives of this research were to 1) investigate the adsorption kinetics and isotherm of cellulase and β-glucosidase on Avicel, dilute acid pCWR and lignin residue of pCWR, and determine the adsorption isotherm parameters; and 2) evaluate the effects of Tween 20 on the adsorption kinetics and isotherm of enzymes on these three substrates.

2. Materials and methods

2.1. Materials

The Creeping Wild Ryegrass (CWR) was planted on the farms located in the San Joaquin Valley of California to help mitigate the salinity problem in soil and drainage irrigation water. The CWR straw was harvested from the Red Rock Ranch at the Five Points in California. The straw was milled into particles using a laboratory hammer mill (Model C269OYB, Franklin Co. Inc., Buffton, IN) equipped with a 0.32-cm rejection screen. After milling, the straw particles were stored in sealed 2-gal zip-loc plastic bags at 4 °C until use. Avicel PH101 (microcrystalline cellulose) and Tween 20 were purchased from Sigma (Sigma, St. Louis, MO). Protein assay kit was purchased from Bio-Rad Laboratory (Bio-Rad, Hercules, CA). The enzymes used in this research included cellulase (Celluclast 1.5 L from *Trichoderma reesei*) and β-glucosidase (Novozymes 188 from *Aspergillus niger*). They were provided by Novozymes Inc. (Davis, CA) as gifts. Cellulase and β-glucosidase had respective activities of 90 FPU/mL and 490 CBU/mL, corresponding to 54 and 65 mg protein/mL, respectively.

2.2. Dilute sulfuric acid pretreatment of creeping wild ryegrass

Raw CWR straw particles were initially pretreated with dilute sulfuric acid (1.4%, w/w) at 165 °C for 8 min in a 1 L Parr reactor (Carpenter 20 Cb-3, Parr Co., Moline, IL) equipped with impeller mixers and a pressurized injection device. Due to the mixing limitation of the Parr reactor, only 10% (w/w, dry basis) solid loading was investigated. The pretreated CWR slurry was thoroughly washed with hot deionized (DI) water at 85 °C to remove the soluble compounds. A portion of the washed pretreated solid was stored at −20 °C for subsequent research. The remaining solid was dried at 45 °C in an oven for chemical composition analysis.

2.3. Preparations of lignin residue

Lignin residue was prepared by a complete batch enzymatic