Kinetics of Adsorption, Desorption, and Re-Adsorption of a Commercial Endoglucanase in Lignocellulosic Suspensions

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ABSTRACT: This study conducted quantitative kinetic modeling and in situ and temporally resolved measurements of adsorption, desorption, and re-adsorption of a commercial endoglucanase in lignocellulosic suspensions. The study defined a cellulase adsorption and desorption competition parameter, a pseudo rate of binding and desorption, binding and desorption capacity, as well as cellulase-binding reversibility (a thermodynamic property) and recyclability (an engineering parameter). The results indicate that both substrate chemical and physical structures play important roles in cellulase binding and desorption. Binding of a commercial cellulase onto a cellulosic substrate was reversible. Bindings to two different lignocellulosic substrates were almost irreversible. While lignin and its structure positively affect binding capacity to substrate, they negatively affect cellulase recyclability. Collapsing of substrate pores reduced cellulose accessibility and cellulase-binding capacity and increased reversibility and recyclability. Increasing temperature and pH increase cellulase desorption and increased binding reversibility and capacity. This study lays the foundation for developing effective cellulase recycling strategies. Biotechnol. Bioeng. 2012;109: 1965–1975. © 2012 Wiley Periodicals, Inc.

KEYWORDS: cellulase-binding kinetics; binding reversibility; adsorption; desorption; enzyme recycling; enzymatic hydrolysis of lignocellulososes

Introduction

Cellulase enzymes have many applications in processing lignocellulosic biomass, such as enzymatic saccharification for sugar/biofuel production (Lynd et al., 2008; Zhu and Pan, 2010), and pretreatment for fiber and nanocellulose production to reduce processing energy consumption (Hart et al., 2009; Henriksson et al., 2007; Pääkkö et al., 2007; Sabourin and Hart, 2010; Spence et al., 2011; Zhu et al., 2011b). However, the cost of cellulase enzymes is still a concern for many commercial applications. This is especially true for the production of commodity products such as sugar/biofuel (Lynd et al., 2008). Many strategies, including increasing substrate reactivity through pretreatment (Kumar et al., 2011; Zhu et al., 2009a) and enzyme recycling (Mes Hartree et al., 1987; Qi et al., 2011; Tu et al., 2007b) have been studied to reduce enzyme cost in sugar/biofuel production from lignocelluloses. These studies suggest that improving substrate digestibility through pretreatments is effective but not without adverse effects, such as increasing cost and the formation of fermentation inhibitors. Further improvement by pretreatment is difficult. Post-pretreatment results in additional processing cost (Kumar et al., 2011). However, recycling of cellulase enzymes can be a potential avenue to save enzyme cost (Qi et al., 2011; Tu et al., 2007a). Desorption of cellulase enzymes from cellulosic or lignocellulosic substrates is a prerequisite step for effective enzyme recycling. Furthermore, the desorbed cellulase needs to be re-adsorbed onto fresh substrates to complete the cellulase recycling.
cycle. Therefore, the studies of desorption and desorption kinetics, re-adsorption, and re-adsorption kinetics of cellulase enzymes in lignocellulosic suspensions under various conditions are important to provide fundamental understanding of the limit of cellulase enzyme recycling.

Several studies examined the effects of pH, temperature, ionic strength, desorber, and solvent on cellulase desorption efficiency (Otter et al., 1989; Seo et al., 2011; Tu et al., 2009; Wu et al., 2010; Zhu et al., 2009b). An early study found that the binding of celllobiohydrolase of Trichoderma reesei to crystalline cellulose was fully reversible (Linder and Teeri, 1996). However, recent studies suggest that the binding of cellulase enzymes in lignocellulosic suspensions under various conditions are important to provide fundamental understanding of the limit of cellulase enzyme recycling.

Desorption ratios of a commercial fungal cellulase enzyme between 10% and 85% from Avicel under various desorption conditions were also reported recently (Zhu et al., 2009b). The desorption of cellulase from lignocellulosic substrates are more complex than from pure cellulose due to the presence of lignin and other noncellulosic components that also have affinity to cellulase (Mansfield et al., 1999; Qing et al., 2010; Sewalt et al., 1997). This affinity varies with lignin structure (Liu et al., 2010; Sewalt et al., 1997). Reported studies on cellulase desorption from lignocellulosic biomass were all substrate specific. Desorption ratios of a commercial fungal cellulase between 5% and 95% from an organic solvent pretreated corn stover were reported (Zhu et al., 2009b). Several studies reported the potential of enzyme recycling from enzymatic hydrolysis of lignocelluloses (Qi et al., 2011; Seo et al., 2011; Tu et al., 2007a, 2009); however, few studies reported the fundamentals on the dynamic competitive phenomenon between adsorption and desorption during cellulase binding to lignocelluloses.

This study has two objectives: (1) to conduct in situ and temporally resolved time-dependent adsorption, desorption, and re-adsorption of a commercial endoglucanase in a cellulose and two pretreated lignocellulosic substrate suspensions under different pH and temperatures; (2) to develop a kinetic cellulase-binding model to describe the binding process and to provide fundamental understanding of the competing physical and biological processes in enzyme-lignocellulose suspensions. The goal of this study is to lay the foundation for developing effective enzyme recycling strategies.

Theory—Cellulase-Binding Model

Cellulase adsorption and desorption are two major competing processes during cellulase binding onto lignocellulosic biomass substrates. The adsorbed cellulase can be bound to solid cellulose substrates by the cellulose-binding module (CBM) or by nonspecific (and nonproductive) association with lignin. The catalytic domain (CD) of cellulase is responsible for randomly cleaving β-1,4-glucosidic bonds of cellulose to shorten cellulose chain length and produce reducing cellulose ends. The terms “binding” and “adsorption” have been used interchangeably in the literature with the assumption of 100% binding efficiency of adsorbed cellulase by the CBM. This study uses “binding” to represent “net adsorption” to differentiate “adsorption.” Furthermore, this study models the total binding and does not differentiate productive and nonproductive binding. As the first step towards comprehensive modeling, the model established here assumes hydrolysis reaction is negligible, and therefore binding sites do not change. This assumption is valid as only endoglucanase is used and most experiments were conducted at 23°C. When a suspension of a lignocellulosic substrate with cellulase is under shear mixing, as in most enzymatic hydrolysis conditions, cellulase diffusion processes can be neglected (Sprague et al., 2004). Therefore, cellulase binding onto lignocellulosic solid substrate can be simplified using the following equation (Sprague et al., 2004),

\[
\frac{df}{dt} = k_{on} \times S \times f - k_{off} \times c
\]

where \( f \) and \( c \) are the normalized concentration of free and bound cellulase, respectively, \( S \) is the number of available binding sites and can be treated as constant when hydrolysis is negligible, and \( k_{on} \) and \( k_{off} \) are the rate constants of cellulase adsorption and desorption, respectively. Neglecting diffusion, free cellulase \( f \) can be expressed as

\[
f = 1 - c
\]

Substitute Equation (2) into Equation (1) with the definition of a pseudo rate constant \( k^*_i = k_{on} \times S \),

\[
\frac{dc}{dt} = k^*_i - (k^*_i + k_{off})c
\]

For adsorption: \( c(t = 0) = 0 \)

For desorption: \( c(t = 0) = 1, \) or \( f = 0 \)

Solving differential Equation (3),

For adsorption:

\[
c = \frac{k^*_i}{k^*_i + k_{off}} \{1 - \exp[-(k^*_i + k_{off})t]\}
\]

For desorption:

\[
f = 1 - c = \frac{k_{off}}{k^*_i + k_{off}} \{1 - \exp[-(k^*_i + k_{off})t]\}
\]
Equation (4a) is different from the expression for the equilibrium state reported in the literature (Moran-Mirabal et al., 2011).

We define an adsorption and desorption competition parameter as follows,

$$
\gamma = \frac{k_{on}^e}{k_{off}}
$$

From Equation (4a) the equilibrium amount \((t \to \infty)\) of bound cellulase onto a substrate during a binding experiment, or the binding capacity, \(C_{eq}\) can be expressed as

$$
C_{eq} = \frac{k_{on}^e}{k_{on}^e + k_{off}} \times C_0 = \frac{\gamma}{1 + \gamma} \times C_0
$$

From Equation (4b), the equilibrium amount \((t \to \infty)\) of net desorbed (free) cellulase during an desorption experiment, or the desorption capacity, \(F_{eq}\) can be written as

$$
F_{eq} = \frac{k_{off}}{k_{on}^e + k_{off}} \times C_0 = \frac{1}{1 + \gamma} \times C_0
$$

where \(C_0\) is the initial amount of cellulase applied in the system either in the suspension as free cellulase (adsorption) or on the substrate as bound cellulase (desorption). Equations (6a) and (6b) suggest that the binding and desorption capacity of a substrate for a given cellulase is directly related to the binding competitive parameter \(\gamma\) and the initial cellulase concentration applied in the system. Furthermore, \(C_{eq}\) increases and \(F_{eq}\) decreases as their respective \(\gamma\) increases. Therefore, a high \(\gamma\) value is desired to bind as much as cellulase as possible for enzymatic hydrolysis; however, a low \(\gamma\) is desired to desorb as much cellulase as possible for cellulase recycling. When \(\gamma = 1\), only half of the amount of cellulase applied can be bound or desorbed.

Cellulase-binding reversibility, \(R_b\), a thermodynamic property, can be defined as the amount of cellulase desorbed after a series (or an infinite number) of desorption (or washing) cycles relative to the amount bound through adsorption. As an approximation, we can define binding reversibility simply as the desorbed fraction after the first desorption, that is, \(R_b = F_{eq}/C_{eq}\). We can also define cellulase recyclability, \(R_o\), an engineering parameter, as the amount of cellulase desorbed after the first desorption plus the amount free cellulase in the filtrate from adsorption relative to the initial amount of cellulase applied in adsorption, that is, \(R_o = (F_{eq} + C_0 - C_{eq})/C_0\). Because adsorption is followed by desorption to recycle bound cellulase, the initial cellulase applied at the beginning of desorption, \(C_{0-de}\), is the equilibrium amount of cellulase adsorbed prior to desorption, or the substrate \(C_{eq}\).

Therefore, we have from Equations (6a) and (6b),

$$
R_b = \frac{F_{eq}}{C_{eq}} = \frac{1}{1 + \gamma_{de}}
$$

$$
R_o = \frac{F_{eq} + C_0 - C_{eq}}{C_0} = \left(\frac{\gamma_{ad} + 1}{1 + \gamma_{de}}\right) \frac{1}{1 + \gamma_{ad}}
$$

where \(\gamma_{de}\) and \(\gamma_{ad}\) denote the competitiveness parameters obtained from first desorption and initial adsorption experiments, respectively. Equation (7) indicates that \(R_b\) is inversely proportional to the competition parameter \(\gamma_{de}\) in desorption. Equation (8) indicates that \(R_o\) increases as either \(\gamma_{de}\) or \(\gamma_{ad}\) decrease. Complete cellulase recycling, that is, \(R_o = 1\), can only be achieved if there is no adsorption, that is either \(\gamma_{ad} = 0\) or \(\gamma_{de} = 0\), or if the substrate is washed infinitely.

We can also define the following overall pseudo rate of binding (net adsorption), \(K_B\), and pseudo rate of net desorption, \(K_D\), as the equilibrium amount of bound cellulase \(C_{eq}\) and desorbed cellulose \(F_{eq}\) divided by a characteristic cellulase binding or desorption time \(\tau = 1/(k_{on}^e + k_{off})\), respectively.

$$
K_B \left(\frac{mg}{g \text{ substrate} \times s}\right) = C_{eq} = k_{on-de} \times C_0
$$

$$
K_D \left(\frac{mg}{g \text{ substrate} \times s}\right) = F_{eq} = k_{off-de} \times C_{0-de}
$$

Equations (9a) and (9b) indicate that \(K_B\) and \(K_D\) depend on the adsorption rate constant in binding or desorption rate constant in desorption, \(k_{on-de}\) and \(k_{off-de}\), respectively, as well as the initial amount of cellulase applied.

Materials and Methods

Materials

Fibercare®, a commercial monocomponent endoglucanase, was generously provided by Novozymes (Franklinton, NC) and used as received. This cellulase was selected because this is an easily accessible monocomponent cellulase. A monocomponent preparation facilitated kinetic modeling. Bio-Rad (Bradford) protein assay kit was obtained from Bio-Rad Laboratories (Hercules, CA). Bovine serum albumin (BSA) was used as standard to calibrate the protein content using the Bradford method (1976). The protein concentration of Fibercare® was 6.4 mg/mL and cellulase activity of 7.5 FPU/mL. All other chemicals used were of ACS reagent grade from Sigma–Aldrich (St. Louis, MO).

Two bleached eucalyptus pulps (BEP) and two lignocellulosic substrates were used. BEP was a never dried BEP. Two bleached eucalyptus pulps (BEP) and two lignocellulosic substrates were used. BEP was a never dried BEP.
from Aracruz Cellulose (Aracruz City, Brazil). Substrate bleached eucalyptus dry lap pulp (BEP-DL) was produced by soaking commercial dry lap pulp board from the same source (Aracruz Cellulose) overnight in deionized water and disintegrated in a lab disintegrator (TMI, Ronkonkoma, NY) for 10,000 revolutions. Substrate bleached eucalyptus never dried pulp (BEP-ND) was a virgin pulp and used to produce a set of hornified substrates by oven drying (od) at 105°C for 2, 8, or 12 h and at 150°C for 3 or 8 h, respectively.

The two lignocellulosic substrates, NE2-DA and NE2-SP, were produced from a hybrid poplar (P. deltoides Bartr. ex Marsh × P. nigra L. “NE222”) by disk milling wood chips after dilute acid (DA) pretreatment and Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulos (SPORL; SP; Zhu et al., 2009a), respectively. NE222 wood chips of 2 kg in od weight were pretreated in a laboratory wood pulping digester of 23 L at 170°C for 25 min. The pretreated wood chips were then disk milled with the addition of water under atmospheric conditions using disk plate gap of 0.25 mm as described elsewhere (Zhu et al., 2011a). Sulfuric acid and sodium bisulfite charges on od weight wood chips were 1.1% and 0%, 1.1% and 2%, for DA and SPORL pretreatment, respectively. The collected solids after disk milling were dewatered by vacuum pressing in a canvas bag. The solid material was then thoroughly washed using deionized water at 50°C. The chemical compositions of the resultant solid substrates were analyzed by the Analytical Chemistry and Microscopy Lab of the USDA Forest Products Laboratory (Table I).

**Water Retention Value (WRV) and Degree of Hornification (DH)**

The WRV of a substrate is a measure of the total substrate pore volume. DH represents the degree of physical structural change of a substrate, that is, pore collapsing, through drying. WRV was determined following SCAN-C 62:00(SCAN-2000). As describe in our previous study (Luo and Zhu, 2011; Luo et al., 2011), approximately 1 g pulp was dispersed in 1-L deionized water and disintegrated in a lab disintegrator for 10,000 revolutions. The resultant suspension was carefully filtered in a Büchner funnel with a 20 μm mesh opening nylon screen (Spectra/Mesh® Nylon, Spectrum Laboratories Inc, Rancho Dominguez, CA). The weight of pulp cake was maintained at 9–11 g. The cake was wrapped in a nylon screen with mesh opening of 100 μm (Cole Parmer, Vernon Hills, IL) and centrifuged at 3,000g for 15 min in a laboratory centrifuge (Thermo Fisher Scientific, Sorvall legend 40/40R Waltham, MA). WRV is the amount of water retained after centrifuge as a percentage of the dry weight of substrate, that is

\[
\text{WRV} = \frac{w_{\text{wet}} - w_{\text{dried}}}{w_{\text{dried}}} \times 100\% \quad (10)
\]

where \(w_{\text{wet}}\) and \(w_{\text{dried}}\) are the wet and dry weights of the substrate, respectively.

Degree of hornification (DH) was defined using the following expression (Luo et al., 2011; Wang et al., 2012),

\[
\text{DH} = \frac{\text{WRV}_{\text{ND}} - \text{WRV}}{\text{WRV}_{\text{ND}} - \text{WRV}_{\text{CD}}} \times 100\% \quad (11)
\]

where \(\text{WRV}_{\text{ND}}\) represents the WRV of the never dried substrate and \(\text{WRV}_{\text{CD}}\) represents the WRV of the complete hornified substrate (for 8 h at 150°C).

**Cellulase Adsorption**

The cellulase adsorption, desorption, and re-adsorption experiments were conducted according to the block flow diagram schematically shown in Figure 1. Adsorption experiments were conducted at lignocellulosic solid substrate consistency of 1% and a cellulase loading of 16 mg protein/g substrate (160 mg protein/L) at 23°C in 50 mM

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**Table 1.** Chemical compositions (wt.%) of the four solid substrates studied.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>K. lignin</th>
<th>Arabinan</th>
<th>Galactan</th>
<th>Glucan</th>
<th>Xylan</th>
<th>Mannan</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEP-DL</td>
<td>0.7</td>
<td>n.d.</td>
<td>n.d.</td>
<td>73.48</td>
<td>15.25</td>
<td>n.d.</td>
</tr>
<tr>
<td>BEP-ND</td>
<td>1.2</td>
<td>0.01</td>
<td>0.01</td>
<td>81.91</td>
<td>5.70</td>
<td>0.02</td>
</tr>
<tr>
<td>NE2-DA</td>
<td>29.6</td>
<td>n.d.</td>
<td>n.d.</td>
<td>61.76</td>
<td>2.05</td>
<td>0.74</td>
</tr>
<tr>
<td>NE2-SP</td>
<td>29.1</td>
<td>n.d.</td>
<td>n.d.</td>
<td>59.00</td>
<td>3.60</td>
<td>1.18</td>
</tr>
</tbody>
</table>

n.d., not detectable.

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**Figure 1.** A schematic flow diagram illustrating cellulase adsorption, desorption, and re-adsorption experiments in suspensions of lignocelluloses. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/bit]
acetic buffer of pH 4.8. The loading of 16 mg protein/g substrate is considered to be a typical dosage for enzymatic hydrolysis of lignocelluloses. The cellulase-binding study onto the BEP-DL substrate was conducted in a range of cellulase loadings of 0–160 mg protein/g substrate, corresponding to 0–160 mg protein/L, at 23°C and pH 4.8. Substrate suspension was well mixed with a magnetic stir bar at 200 rpm. The suspension of cellulase and lignocellulosic substrate was circulated to a quartz flow cuvette (1.5 mL) using a peristaltic pump (Model M312, Gilson, Middleton, WI) and a flow loop consisting of an Erlenmeyer flask and a Teflon tube. A stainless steel tube screen of 400 mesh was wrapped at the inlet of the Teflon tube to filter fibers. Absorption spectra of free cellulase in the suspension were recorded every 10 s for 90 min using a UV–Vis spectrophotometer (model 8453; Agilent Technologies, Palo Alto, CA).

The amount of cellulase adsorbed onto a solid substrate was determined as the difference between the total amount of cellulase initially applied and the amount of free cellulase in the solution. The amount of free cellulase (protein) in the solution was measured by an in situ and rapid UV–Vis spectrophotometric technique (Liu et al., 2011). The technique determines free protein concentration in the solid substrate suspension from the second derivative of the absorption spectra at 291 nm with respect to wavelength through calibration. The interferences from light scattering and absorptions from other species, mainly fines and leached lignin, on endoglucanase absorption was eliminated using this spectral derivative method.

**Filtration, Desorption, and Re-Adsorption**

After adsorption, the substrate suspension was filtrated using a Büchner funnel under vacuum with a 20-μm mesh opening nylon screen (Spectra/Mesh® Nylon, Spectrum Laboratories Inc.). The filtrate was recycled two times to avoid losses of fines. The final filtered cake was approximately 2.6 g in wet weight, corresponding to a solid content of 38%. The collected solid substrate cake was used for the desorption study. The collected filtrate was used for the re-adsorption study using corresponding fresh substrate.

Desorption of cellulase from the collected solid substrate was conducted in a 50 mM acetic buffer with pH adjusted to 3.2, 4.8, and 7.2 at 23 and 50°C, and in a 100 mM acetic buffer at pH 4.8 and 23°C (Fig. 1). The substrate cake was added into an Erlenmeyer flask to make a 100 mL suspension well mixed with magnetic stirrer at 200 rpm. Again the time-dependent free cellulase concentrations in the suspension were measured by the UV–Vis technique described.

The filtrate from the BEP-DL and NE2-DA substrate adsorption experiment was added with corresponding fresh solid substrate to study re-adsorption. Since the NE2-SP substrate adsorbed almost all of the cellulase leaving only a trace amount of free cellulase in the filtrate, re-adsorption experiment was not conducted. The re-adsorption conditions were pH = 4.8 and 23°C with acetic buffer concentration of 50 mM (Fig. 1). The initial cellulase concentrations were the free cellulase concentrations in the filtrates, that is, 85.6 and 36.8 mg protein/L for the BEP-DL and NE2-DA substrate, respectively.

**Data Analysis**

Duplicate adsorption experiments were conducted to obtain averaged time-dependent adsorption or desorption data. Repeatability of the data was generally good without significant deviations. Nonlinear curve fitting of the time-dependent adsorption and desorption data using Equations (4a) and (4b), respectively, were conducted using a commercial graphic software Origin 8.1 (Origin Lab Corporation, Northampton, MA). To demonstrate the sensitivity of the model in response to errors in measured adsorption or desorption, we plotted two sets of predicted time-dependent adsorption values (dotted lines in Fig. 2) from the model (Eq. 4a) using ±10% deviation in $k_{\text{on}}$ and $k_{\text{off}}$, that is, $(0.9k_{\text{on}}; 1.1k_{\text{off}})$ and $(1.1k_{\text{on}}; 0.9k_{\text{off}})$, respectively. The results indicate that the difference in substrates (signal) is much larger than the random error (noise) except for experiment with very small $k_{\text{off}}$ (<1) using sample NE2-SP. Similar sensitivity behavior can also be observed from the fitting standard errors of $k_{\text{on}}$ and $k_{\text{off}}$ as listed in Tables II–IV.

**Results and Discussions**

**Time-Dependent Adsorption of Cellulase Onto Lignocellulosic Substrates**

Cellulase binding depends on the properties of the substrates as illustrated by the time-dependent amounts of bound...
cellulase onto different substrates (Fig. 2). The cellulosic substrate BEP-DL bound significantly less cellulase than the two lignocellulosic substrates, NE2-DA and NE2-SP. The differences in the amounts of bound cellulase onto NE2-DA and NE2-SP, produced using different pretreatment methods, were also very different even though the chemical compositions of the two substrates were similar (Table 1). This suggests that lignin sulfonation by SPORL pretreatment may play a role in facilitating cellulase binding. Under the cellulase loading of 16 mg/g substrate, NE2-SP bound almost all the cellulase applied in the suspension.

The measured time-dependent cellulase-binding data agree very well with the cellulase-binding model (Eq. 4a) as shown in Figure 2. The equilibrium amounts of bound cellulase, rates of binding, $K_B$, and adsorption and desorption rate constants, $k^*_\text{on-ad}$ and $k^*_\text{off-ad}$, along with competitive measures, $\gamma_{\text{ad}}$, between adsorption and desorption were determined by fitting the experimental data using Equation (4a). As listed in Table II, cellulase adsorption and desorption rate constants $k^*_\text{on-ad}$ and $k^*_\text{off-ad}$, and binding rate, $K_B$, vary with substrate. BEP-DL has the lowest adsorption rate constant while the NE2-SP has the highest. The rate constant $k^*_\text{on-ad}$ is more than two orders of magnitude greater than the desorption rate constant $k^*_\text{off-ad}$ that is, $\gamma_{\text{ad}} = 122$, for NE2-SP. However, $\gamma_{\text{ad}} = 3.3$ for NE2-DA produced from the same poplar wood with DA pretreatment. Both substrates bind more than half of the available cellulase because $\gamma_{\text{ad}} > 1$. By comparison, further binding (net adsorption) cannot take place when the amount of free cellulase is lower than the amount cellulase bound for BEP-DL as $\gamma_{\text{ad}} = 0.8 < 1$. These observations suggest that lignin binds cellulase and lignin structure may play a role in this binding. This agrees with literature findings on nonproductive cellulase binding onto lignin (Liu et al., 2010; Mansfield et al., 1999; Sewalt et al., 1997).

### Table III

<table>
<thead>
<tr>
<th>Substrate</th>
<th>pH</th>
<th>T (°C)</th>
<th>$C_{\text{in-de}}$ (mg/g substrate)</th>
<th>$k^*_{\text{on-de}} \times 10^4$ (1/s)</th>
<th>$k^*_{\text{off-de}} \times 10^3$ (1/s)</th>
<th>$K_D \times 10^4$ (mg/g substrate/s)</th>
<th>$\gamma_{\text{de}}$</th>
<th>$F_{\text{eq}}$ (mg/g substrate)</th>
<th>Model fitting $r^2$</th>
<th>Desorbed fraction or binding reversibility, $R_b = \frac{f_{\text{de}}}{f_{\text{eq}}}$</th>
<th>Cellulase recyclability, $R_b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEP-DL</td>
<td>4.8</td>
<td>23</td>
<td>38.2 ± 1.22</td>
<td>7.3 ± 1.22</td>
<td>54.0</td>
<td>5.26</td>
<td>1.19 ± 0.005</td>
<td>0.915</td>
<td>0.160</td>
<td>0.610</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>4.8*</td>
<td>23</td>
<td>37.1 ± 0.99</td>
<td>7.3 ± 0.99</td>
<td>54.2</td>
<td>5.07</td>
<td>1.22 ± 0.004</td>
<td>0.941</td>
<td>0.165</td>
<td>0.613</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>23</td>
<td>19.3 ± 0.34</td>
<td>9.9 ± 0.34</td>
<td>73.2</td>
<td>1.96</td>
<td>2.51 ± 0.005</td>
<td>0.979</td>
<td>0.338</td>
<td>0.693</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>50</td>
<td>7.4 ± 0.23</td>
<td>13.2 ± 0.23</td>
<td>98.3</td>
<td>0.56</td>
<td>4.77 ± 0.014</td>
<td>0.990</td>
<td>0.643</td>
<td>0.834</td>
<td>0.103</td>
</tr>
<tr>
<td>NE2-DA</td>
<td>4.8</td>
<td>23</td>
<td>22.9 ± 2.97</td>
<td>0.50 ± 2.97</td>
<td>6.2</td>
<td>45.4</td>
<td>0.27 ± 0.011</td>
<td>0.532</td>
<td>0.022</td>
<td>0.247</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>23</td>
<td>48.8 ± 5.33</td>
<td>1.87 ± 5.33</td>
<td>23.0</td>
<td>26.1</td>
<td>0.45 ± 0.013</td>
<td>0.670</td>
<td>0.037</td>
<td>0.258</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>50</td>
<td>52.0 ± 4.46</td>
<td>6.14 ± 4.46</td>
<td>75.7</td>
<td>8.5</td>
<td>1.30 ± 0.043</td>
<td>0.964</td>
<td>0.106</td>
<td>0.311</td>
<td>0.103</td>
</tr>
<tr>
<td>NE2-SP</td>
<td>4.8</td>
<td>23</td>
<td>9.5 ± 1.05</td>
<td>0.25 ± 1.05</td>
<td>3.9</td>
<td>39.0</td>
<td>0.40 ± 0.020</td>
<td>0.737</td>
<td>0.025</td>
<td>0.033</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>23</td>
<td>8.5 ± 0.41</td>
<td>0.38 ± 0.41</td>
<td>6.1</td>
<td>22.2</td>
<td>0.68 ± 0.015</td>
<td>0.942</td>
<td>0.043</td>
<td>0.051</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>50</td>
<td>17.3 ± 1.05</td>
<td>1.42 ± 1.05</td>
<td>22.6</td>
<td>12.2</td>
<td>1.21 ± 0.027</td>
<td>0.921</td>
<td>0.076</td>
<td>0.083</td>
<td>0.103</td>
</tr>
</tbody>
</table>

Acetic acid buffer concentration: 100 mM for the run denoted with “*” and 50 mM for the rest of the runs. The error ranges of $k^*_\text{on-de}$ and $k^*_\text{off-de}$ and $C_{\text{eq}}$ or $F_{\text{eq}}$ are from nonlinear fitting standard errors. The fitting standard errors for $(k^*_\text{on} + k^*_\text{off})$ are used as the error ranges for both $k^*_\text{on}$ and $k^*_\text{off}$. 

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Table IV. Effect of drying-induced substrate hornification on adsorption and desorption rate constants and the equilibrium amounts of adsorption (23°C and pH = 4.8) and desorption (50°C and pH = 7.2) with 50 mM acetic buffer.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>DH</th>
<th>Cellulase loading C₀ (mg/g substrate)</th>
<th>Kₓ⁻→ₓ₀ (s⁻¹)</th>
<th>Kₓ₀⁻→ₓ (s⁻¹)</th>
<th>Kᵦ × 10⁴ (mg/g substrate/s)</th>
<th>Model fitting r²</th>
<th>Bound fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEP-ND</td>
<td>0</td>
<td>7.82 ± 0.14</td>
<td>15.24 ± 0.30</td>
<td>8.86 ± 0.30</td>
<td>69.3 ± 0.30</td>
<td>0.97 ± 0.00</td>
<td>0.36 ± 0.00</td>
</tr>
<tr>
<td>BEP-T10502</td>
<td>0.14</td>
<td>7.07 ± 0.45</td>
<td>15.12 ± 0.45</td>
<td>12.28 ± 0.45</td>
<td>86.9 ± 0.45</td>
<td>1.23 ± 0.01</td>
<td>0.36 ± 0.01</td>
</tr>
<tr>
<td>BEP-T10508</td>
<td>0.56</td>
<td>6.57 ± 0.30</td>
<td>12.41 ± 0.30</td>
<td>11.39 ± 0.30</td>
<td>74.8 ± 0.30</td>
<td>1.09 ± 0.01</td>
<td>0.49 ± 0.01</td>
</tr>
<tr>
<td>BEP-T10511</td>
<td>0.79</td>
<td>6.33 ± 0.25</td>
<td>10.69 ± 0.25</td>
<td>10.41 ± 0.25</td>
<td>65.9 ± 0.25</td>
<td>1.03 ± 0.01</td>
<td>0.49 ± 0.01</td>
</tr>
<tr>
<td>BEP-T10512</td>
<td>0.87</td>
<td>5.98 ± 0.39</td>
<td>7.22 ± 0.39</td>
<td>12.18 ± 0.39</td>
<td>48.4 ± 0.39</td>
<td>0.59 ± 0.01</td>
<td>0.62 ± 0.01</td>
</tr>
<tr>
<td>BEP-T15008</td>
<td>1.00</td>
<td>3.85 ± 0.14</td>
<td>11.65 ± 0.14</td>
<td>11.05 ± 0.14</td>
<td>61.7 ± 0.14</td>
<td>0.33 ± 0.01</td>
<td>0.98 ± 0.01</td>
</tr>
</tbody>
</table>

Substrate label Txxx stands for drying temperature in °C and txx stands for drying duration in hours. The error ranges of Kₓ⁻→ₓ₀, Kₓ₀⁻→ₓ, and Cₓ₀ or Fₓ₀ are from nonlinear fitting standard errors using Equation (4a) or (4b). The fitting standard errors for (Kₓ⁻→ₓ₀ + Kₓ₀⁻→ₓ) are used as the error ranges for both Kₓ⁻→ₓ₀ and Kₓ₀⁻→ₓ.

**Effect of Cellulase Concentration on Adsorption Onto a Cellulosic Substrate**

A similar adsorption study was conducted using BEP-DL in a range of cellulase concentrations (loadings). It was found that the amount of bound cellulase achieved an asymptotic value at cellulase concentration (loading) of 160 mg protein/L (16 mg protein/g substrate) or higher. Therefore, a binding study within this concentration range can show the effect of cellulase concentration on binding process. The results indicate that the fraction of bound cellulase decreased from 0.65 to 0.46 (or by 30%) as cellulase concentration was increased from 20 to 160 mg protein/L, corresponding to free cellulase concentration of approximately 6–86 mg protein/L (Fig. 3). In Figure 3 the x-axis is free protein concentration, therefore the data will be applicable at other fiber dilution levels when expressed this way. Desorption became more competitive as cellulase concentration increased as reflected by the reduction in γₐd from 1.88 to 0.86 (or by 54%). However, the Kᵦ was increased from 0.0026 to 0.0152 mg protein/g substrate/s (or by 584%) because cellulase concentration (loading) was increased by 800% (20–160 mg protein/L). The data indicate that adsorption and desorption are equally competitive (γₐd = 1) at free cellulase concentration approximately 60 mg protein/L (Fig. 3), or total cellulase concentration of 120 mg protein/L based on Equation (6a).

**Time-Dependent Desorption of Cellulase From Lignocellulosic Substrates**

Instantaneous cellulase desorption from lignocellulosic substrates was monitored. The cellulase-binding model, Equation (4b), fits the measured time-dependent desorption very well for cellulose substrate BEP-DL, as shown in Figure 4. The results indicate that pH and temperature significantly affected desorption kinetics. No desorption occurred at pH = 5.2. Both the desorption rate, Kᵦ, and
The equilibrium amount of desorption, $F_{eq}$, increased as pH and temperature were increased for the three substrates studied (Table III, Figs. 4 and 5). The amount of desorption was doubled when pH was increased from 4.8 to 7.2. To avoid cellulase denaturation the maximum pH studied was 7.2. Similarly, the amount of desorption almost doubled when temperature was increased from 23 to 50 °C. However, increasing buffer concentration from 50 to 100 mM had no effect on desorption (the observed differences were within nonlinear fitting standard errors). The effects of pH and temperature on desorption can be best observed from the competition between adsorption and desorption $g_{de}$, and $K_D$, that is, $g_{de}$ decreased from 5.26 at pH = 4.8 and 23 °C to 0.56 at pH = 7.2 and 50 °C, while $K_D$ increased from 0.0054 to 0.0098 mg/g substrate/s. The desorption fractions, or $R_b$, ranged from 0.16 to 0.64, suggesting binding of the commercial endoglucanase on BEP-DL (a cellulosic substrate) is partially reversible based on Equation (7) with $g_{de}$ on the order of unity. More than 50% of the applied cellulase into the system can be recycled (Table III). Cellulase recyclability was over 80% when desorption was conducted at 50 °C and pH 7.2.

Cellulase desorbed much more easily from the cellulosic substrate, BEP-DL, than from the two lignocellulosic substrates, NE2-DA and NE2-SP. This can be clearly seen from $g_{de}$ as well as the fraction of cellulase desorbed (Table III). The competitive parameter $g_{de}$ of NE2-DA and NE2-SP are significantly higher ($\gg 1$) than the corresponding value for BEP-DL under the same desorption conditions. This suggests that binding of the commercial endoglucanase to the two lignocellulosic substrates were almost irreversible based on Equation (7). The maximal desorption (or the reversibility) was only approximately 10% from these two lignocellulosic substrates. These observations also suggest that lignin plays an important role in cellulase desorption, which can limit the recycling of cellulose bound to lignocellulosic substrates. The maximal recyclability, $R_0$, was 31% and 8% for NE2-DA and NE2-SP, respectively, much lower than the 83% achieved from BEP-DL (Table III).

When examining both the binding (Table II) and desorption data (Table III) of the three substrates, it is clear that the higher the adsorption competitive parameter, $g_{ad}$, the higher the $K_B$ and lower the $K_D$ due to reduced rate constant $k_{off-de}$ in desorption. Furthermore, when increasing the desorption rate constant $k_{off-de}$ through increasing temperature or pH in desorption, the rate constant of adsorption $k_{on-ad}$ in desorption was decreased for BEP-DL (adsorption competitive parameter in binding $g_{ad} < 1$) and
was increased for NE2-DA and NE2-SP (binding $\gamma_{ad} > 1$). This further illustrates the inherent difference in cellulase binding and desorption among different substrates, that is, a substrate, such as NE2-SP, with higher binding competitive parameter, $\gamma_{ad}$, tends to desorb less cellulase than a substrate with lower binding $\gamma_{ad}$ such as BEP-DL. Because cellulase recyclability is the sum of the amount cellulase desorbed and the amount of free cellulase, $C_0 - C_{eq}$ or 1–bound fraction, in the filtrate of binding experiment (Table II), the recyclability of NE2-DA is much higher than that of NE2-SP even though the difference in desorption fraction between these two substrates was not much different (Table III).

Further examining the time-dependent desorption data for NE2-DA and NE2-SP revealed a very interesting phenomenon about the competition between adsorption and desorption (Fig. 5), especially at high pH (7.2) and temperature (50°C). Net desorption ended at approximately 400 and 1,400 s into desorption for the NE2-DA and NE2-SP, respectively. Only the desorption data within the 400 and 1,400 s were used to fit the cellulase-binding model (Eq. 4b) for NE2-DA and NE2-SP, respectively, to obtain the results in Table III. Free cellulase in the system started to rebind to the substrates, which resulted in the reduction of free cellulase concentration (amount of desorbed cellulase). This phenomenon suggests the strong irreversibility of cellulase binding to the two lignocellulosic substrates, which is reflected by the low values of $R_b$ (Table III). The adsorption and desorption environment conditions, such as pH and temperature, can change the competition between adsorption and desorption (Table III). The results in Figures 4 and 5 clearly indicate the importance of kinetic study for effective enzyme recycling, that is, the termination of desorption for BEP-DL, NE2-DA, and NE2-SP should be enacted at different times. It should be noted that in industrial settings, the amount of cellulose substrate will diminish over time. Enzyme release from cellulose will concurrently diminish in importance, but release from nonspecific lignin adsorption will remain a critical parameter.

**Cellulase Re-Adsorption of Recycled Cellulase**

The time-dependent binding of the recycled free cellulase in the filtrates of the BEP-DL and NE2-DA suspensions onto fresh BEP-DL and NE2-DA, respectively, were measured. The results indicate that the binding of the recycled enzymes through re-adsorption to the corresponding fresh substrate was not efficient. $\gamma_{ad}$ values were reduced from 0.87 and 2.35 to 0.26 and 1.04, for BEP-DL and NE2-SP (Table II), respectively. To account for the effect of initial free cellulase concentration on $\gamma_{ad}$, $K_B$ and bound fraction, comparisons of these three parameters between binding experiments using fresh and recycled cellulase on BEP-DL were made in Figure 3. The results clearly show the inefficient binding of recycled cellulase to corresponding substrate. This is probably due to the fact that the commercial endoglucanase studied may contain different components even when labeled as monocomponent. The binding efficiencies among different component enzymes may be different. The recycled cellulase may contain more enzymes with low-binding efficiencies. The present UV–Vis spectroscopic method only measures the total protein that cannot differentiate different enzymes. The damage of proteins in the recycling process with inefficient binding may also play a role. The high pH 7.2 used in desorption experiment may also contribute by causing cellulase denaturing or reducing stability. Further studies using pure mono-component cellulase is needed to elucidate this point.

**Effects of Substrate Pore Structure on Cellulase Adsorption and Desorption**

Drying of lignocellulosic substrates can cause irreversible collapse of pores due to hydrogen bonding (Luo et al., 2011; Wang et al., 2012). As a result of this limited substrate accessibility, cellulase binding is limited (Wang et al., 2012). Using a set of dried substrates with very different pore structure but identical chemical structure (as they were produced from the same never dried cellulose sample BEP-ND), this study further revealed the effect of pore structure on the competition between adsorption and desorption and the reversibility of cellulase binding. It was found that the fraction of bound cellulase decreased (increasing $R_b$) as the extent of drying increased represented by the DH (Table IV). Desorbed fraction or $R_d$ was increased from 0.37 (DH = 0) to 0.63 (DH = 1). $\gamma_{de}$ values for desorption were greater than 1 (DH < 1) except for the completely hornified substrate (DH = 1), suggesting drying increased the relative importance of desorption, in agreement with our previous studies that reported reduced binding (Luo et al., 2011; Wang et al., 2012). Furthermore, the reduction of $\gamma_{ad}$ (less competitive adsorption) with DH from approximately 1 at DH = 0 to 0.33 at DH = 1 suggesting the collapse of pores reduced cellulase binding (Table IV). The corresponding $C_{eq}$ and $K_B$ were reduced from 7.82 to 3.98 mg/g substrate and 0.0121 to 0.0062 mg/g substrate/s, respectively, or reduced by 49% in both cases. If the only impact of hornification were to reduce the number of binding sites, then the $K_B$ would scale linearly with capacity $C_{eq}$. Since this is the case, we suggest that the major impact of hornification was elimination of cellulase-binding sites. These observations indicate that pore collapse greatly reduces cellulase accessibility and binding capacity.

**Conclusions**

The cellulase-binding model developed in this study quantitatively described cellulase-binding kinetics with good agreement with the temporally resolved experimental data of adsorption, desorption, and re-adsorption of a commercial endoglucanase in three different lignocellulose suspensions under various conditions. The model developed
should be applicable to other cellulase enzymes. The study defined an adsorption and desorption competition parameter $\gamma$ (Eq. 5) and the pseudo rate of binding, $K_B$ (Eq. 9a), and $K_D$ (Eq. 9b). The study also defined cellulase-binding reversibility $R_0$ (Eq. 7), a thermodynamic property, and cellulase recyclability $R_0$ (Eq. 8), an engineering parameter. The binding capacity is proportional to the initial available cellulase and competition parameter $\gamma_{ad}$ in binding. The desorption capacity is also proportional to the available bound cellulase for desorption, but inversely proportional to the competition parameter $\gamma_{de}$ in desorption. The competitive parameter in desorption $\gamma_{de} \ll 1$ and $\gamma_{de} \gg 1$ for fully reversible and irreversible binding. $\gamma_{de} = 1$ for partially (50%) reversible binding. The $R_0$ increases as either $\gamma_{ad}$ or $\gamma_{de}$ decreases.

Lignin and lignin structure play significant roles in cellulase binding, desorption, reversibility, and recyclability. The two lignocellulosic substrates showed significantly higher affinity to the studied cellulase than the bleached pulp, with much higher $\gamma_{ad}$ and $\gamma_{de}$ than those of bleached pulp, respectively. As a result, the reversibility of cellulase-binding to each of the two lignocellulosic substrates is lower than to the bleached pulp. Furthermore, the recyclability of cellulase from each of two lignocellulosic substrates is much lower than from the bleached pulp. Lignin also facilitated the total cellulase adsorption with increased binding capacity and binding rate. Substrate physical pore structure also significantly affects cellulase binding, reversibility, and recyclability but not desorption. Drying induced fiber hornification reduced cellulase binding sites or cellulose accessibility to cellulase and therefore reduced cellulase-binding capacity, but increased binding reversibility and cellulase recyclability. The study also found that increasing temperature and pH increased desorption for all three substrates studied and are therefore favorable for cellulase recycling.

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**Nomenclature**

$c$ normalized concentration of bound cellulase

$f$ normalized concentration of free cellulase

$k_{ad}$ rate constant of cellulase desorption

$k_{on}$ rate constant of cellulase adsorption

$k_{on} = k_{on} \times S$

$S$ available binding sites

$\gamma = \frac{k_{on}}{k_{ad}}$ adsorption desorption competition parameter

$C_0$ initial amount of cellulase applied in the system either in the suspension as free cellulase (adsorption) or on the substrate as bound cellulase (desorption, mg/g substrate)

$C_{eq}$ binding capacity (mg/g substrate)

$F_{eq}$ desorption capacity (mg/g substrate)

$K_B = k_{on} \times C_0$ binding rate (mg/g substrate/s)

$K_D = k_{off} \times C_0$ desorption rate (mg/g substrate/s)

$R_0 = \frac{F_{eq} \times C_0}{C_{eq}}$ cellulase recyclability

$R_0 = \frac{k_{on} - k_{off}}{k_{on}}$ binding reversibility

**Subscripts**

$ad$ adsorption

$de$ desorption

**References**


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