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Enzymatic hydrolysis, adsorption, and recycling during hydrolysis of bagasse sulfite pulp

Jia Ouyang [a,*], Baotian Liu [b], Min Zhang [b], Zhaojuan Zheng [b], Heng Yu [b]

[a] Jiangsu Key Lab of Biomass-Based Green Fuels and Chemicals, Nanjing 210037, People's Republic of China
[b] College of Chemical Engineering, Nanjing Forestry University, Nanjing 210037, People's Republic of China

HIGHLIGHTS

- Bagasse sulfite pulp showed a good performance on enzymatic hydrolysis.
- Cellulase adsorption isotherm for the fresh BSP was well fitted to a Sips model.
- The cellulase obtained by re-adsorption exhibited the higher efficiency.
- A relatively simple strategy for enzyme recycling is proposed.

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ABSTRACT

The high costs of enzymatic hydrolysis along with the high enzyme dosage are often considered as the major bottlenecks in lignocellulosic bioconversion. This study investigated the hydrolysis efficiency, cellulase adsorption and enzyme recycling during the hydrolysis of bagasse sulfite pulp (BSP). After 48 h of hydrolysis, more than 70% of the cellulose was hydrolyzed, while the protein concentration and cellulase activity in solution remained 31% and 17% of the initial value, respectively. The cellulase adsorption on the fresh BSP was better fitted by a Sips model, suggesting the occurrence of a multilayer adsorption at low cellulase concentration and monolayer adsorption at high concentration on the BSP surfaces. Desorption profile studies showed that the optimum desorption condition was at pH 4.8 and 40 °C. Moreover, considering the limited ability to desorption, directly empolying the bound enzyme with residual substrate is more effective method to recover cellulase during the hydrolysis of BSP.

1. Introduction

Currently, the utilization of lignocellulose biomass as a feedstock for microbial production of bulk biochemical has attracted extensive attention. Many studies have been conducted on this topic. But due to the complex cell-wall structure of lignocellulose biomass, enzymatic conversion of lignocellulose biomass into fermentable sugars is still the major obstacle to economical production. Many factors have been found to contribute to the high cost of enzymatic hydrolysis, such as enzyme inhibition, substrate accessibility to enzymes, or nonproductive binding of enzyme to lignin (Qing et al., 2010; Leu and Zhu, 2013). For this reason, various methods were developed to improve the enzyme performance by increasing enzyme specific activity and preventing nonspecific adsorption of enzymes (Tu et al., 2009; Tu and Saddler, 2010). However, until now, an efficient enzymatic hydrolysis process still requires high enzymatic dosages which result in the high enzyme prices. Therefore, a significant amount of effort has been devoted to the recycling of cellulase enzyme.

After enzymatic hydrolysis of lignocellulose, the enzymes are distributed between the liquid phase and the solid phase residues (Pribowo et al., 2012; Eckard et al., 2013). Hence, there have been two strategies to recover cellulases. Free cellulase in bulk solution could be recovered by adding fresh substrate or ultrafiltration (Qi et al., 2011, 2012). Compared with ultrafiltration, adding free cellulosic substrate is an easy and inexpensive method to recover free cellulase from the liquor. Recently, several studies have confirmed that free cellulase in bulk solution could be recovered by means of addition of fresh substrate. However, enzyme adsorption is mainly influenced by the properties of cellulosic substrates. Alkali treated wheat straw behaved lower affinity to cellulase than acid treated substrate (Qi et al., 2011). A consistently positive correlation was found between adsorption and available specific surface area for the SO_2 catalyzed steam-pretreated wheat straw materials (Piccolo et al., 2010). In most cases, the enzyme adsorption follows the

* Corresponding author. Address: College of Chemical Engineering, Nanjing Forestry University, Nanjing 210037, People's Republic of China. Tel.: +86 025 85427587; fax: +86 025 83587330.
E-mail addresses: hgouyj@njfu.edu.cn, Jia_ouyang72@hotmail.com (J. Ouyang).

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Langmuir isotherm, which suggests that cellulases are adsorbed onto lignocellulose as forming monomers (Qi et al., 2011). As for the enzymes bound to the residual substrate, they could be directly reused in the 2nd hydrolysis (without desorption) (Lee et al., 1995). But some authors reported that this strategy would result in an increased build up of lignin rich residues, which had a significant adverse effect on the hydrolytic ability of the recovered enzyme (Lee et al., 1995; Qi et al., 2011; Rodrigues et al., 2012). Hence, other research had been devoted to desorbing the bound enzyme using alkaline elution or adding surfactants (Rodrigues et al., 2012; Seo et al., 2011). Moreover, during the hydrolysis of ethanol pretreated mixed softwood, it was found that cellulase desorption from the hydrolysis residues appeared better recycling efficiency than without a desorption treatment (Tu et al., 2009).

Although a significant amount of efforts had been devoted to recover the enzymes by enzyme adsorption, desorption, and re-adsorption on lignocellulosic, developing an efficient enzyme recycling still remains a challenge required to be addressed. Enzyme adsorption and desorption are known to be highly dependent on the nature of substrate (Wang et al., 2012), resulting in different recycling performance (Eckard et al., 2013). Moreover, as of our knowledge, few studies have yet evaluated the hydrolysis efficiency of the bound or free enzyme after recycling process. Therefore in this work, cellulase adsorption on fresh substrate and desorption from hydrolysis residues were studied for enzyme recycling. On the basis of these results, several recovering enzyme methods, whether from liquid phase or solid phase were evaluated to determine their efficiencies during the sequent hydrolysis of bagasse sulfite pulp (BSP).

2. Methods

2.1. Substrates

BSP was kindly provided by Jiangmen Sugar Cane Chemical Co., Ltd (Jiangmen, China). They were washed by 10-fold distilled water (w/w), filtered, and stored in sealed plastic bags at 4 °C. The compositions of BSP were analyzed according to NREL procedure "determination of structural carbohydrates and lignin in biomass" (NREL/TP-510-42618) (Sluiter et al., 2006). Physical properties of BSP were determined by the Brunauer–Emmett–Teller (BET) method (Piccolo et al., 2010). Surface area and total pore volumes were determined at a relative pressure of about 0.99 on a Micromeritics ASAP 2020 (Micromeritics Instrument Corporation, USA) after out gassing for 10 days at 40 °C. Before degassing, the materials were dried for more than 24 h in a vacuum oven at 35 °C.

2.2. Enzymes

As the cellulase solution, Celluclast 1.5L produced by *Hypocrea jecorina* (Cat C2730, Lot 058K1200) was used for all experiments. In addition, during the hydrolysis of BSP, β-glucosidase from *Aspergillus niger* (Novozyme 188, Cat C6105, Lot 097K0682) was supplemented to reduce the cellobiose inhibition. These two commercial enzyme solutions were both purchased from Sigma–Aldrich. The enzyme activity of Cellulast 1.5L and Novozyme 188 were described as filter paper activity units (FPU) and cellobiase units (CBU), respectively.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was performed at 8% (w/v) cellulose concentration in 30 mL of citrate buffer (50 mM, pH 4.8) on a thermostated shaker set at 50 °C and 150 rpm for 48 h. The total working volume was 30 mL in 100 mL Erlenmeyer flasks. A Cellulast 1.5L loading of 15 FPU/g cellulose with the addition of Novozyme 188 at a loading of 15 CBU/g cellulose was used for all hydrolysis experiments. At specific time intervals, aliquots were withdrawn and centrifuged to remove the insoluble materials. The supernatants were subsequently filtered through a 0.22 μm syringe filter (Millipore, Bedford, MA) and used for subsequent analysis. All experiments were performed in duplicate and results represented the mean values of two independent experiments.

2.4. Cellulase adsorption on BSP

The adsorption of Cellulast 1.5L was performed on fresh BSP in 25 mL of 50 mM sodium citrate buffer (pH 4.8) at 8% (w/v) cellulose concentration and 1.2 FPU/mL Cellulast 1.5L. Considering BSP is decomposed by cellulase, adsorption experiment of cellulase was performed at 4 °C and 100 rpm to keep the catalytic activity of cellulase negligible (Kumar and Wyman, 2009). Aliquots were taken at different time intervals during the incubation. The samples were centrifugated and the supernatant was collected. The cellulase that remained in the supernatant was determined by the protein assay.

Cellulase adsorption isotherm on BSP was conducted by varying the dosage of cellulase at 8% (w/v) cellulose concentration. The reactions were incubated with shaking at 4 °C for 2 h to reach equilibrium. The amount of cellulase bound to the substrate was calculated as the difference between free enzyme in liquid fraction and the initial enzyme dosage using the protein assay. All experiments were performed in duplicate.

2.5. Desorption of saccharification enzymes from hydrolysis residues

After 48 h hydrolysis of BSP as described in Section 2.3, the mixture was centrifuged at 4000*g* for 15 min. The residues (a solid content of 32%) were collected for desorption study. Desorption of enzymes from the collected solid substrate was conducted at different temperature, pH. About 5 g hydrolysis residue was added into 10 mL buffer to make a well mixed suspension at 100 rpm. After incubation for 2 h at 100 rpm, the mixture was centrifuged at 8000*g* for 10 min and the supernatant with desorbed enzyme was collected for cellulase activity and protein assays. All experiments were performed in duplicate.

2.6. Enzyme recycling experiment

To compare the capability of the enzyme adsorption, desorption and re-adsorption on enzyme recycling, enzyme recycling experiments were carried out according to the schematic diagrams of four methods, which were shown in Fig. 1.

After 48 h of 1st hydrolysis, cellulase recycling was performed by separately collecting the residual substrates and the liquid. In Method A, fresh substrates were mixed with suspension to recover the free enzymes by adsorption. Adsorption experiments were conducted at 4 °C, 4% (w/v) cellulose concentration and 100 rpm for 2 h to recover the free enzyme. As for Method B, the bound enzyme was desorbed from the hydrolysis residues at the optimal conditions, and then was reused for the 2nd hydrolysis. Method C represents a simpler enzyme recycling method where the bound enzyme, existed in the unhydrolyzed residue, was directly recycled in the 2nd hydrolysis (without desorption). As the control of Method C, the obtained solid after the 1st hydrolysis experiment was continually hydrolyzed and no fresh substrates were added in Method D (Ouyang et al., 2009).

The 2nd hydrolysis was conducted to evaluate the effect of these recovered cellulases. The experiments were performed in duplicates at 50 °C, pH 4.8 and 150 rpm for 48 h with 4% (w/v) cellulose concentration. Fresh β-glucosidase was supplemented at a