Science of the Total Environment 647 (2019) 806–813

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effective one-step saccharification of lignocellulosic biomass using magnetite-biocatalysts containing saccharifying enzymes

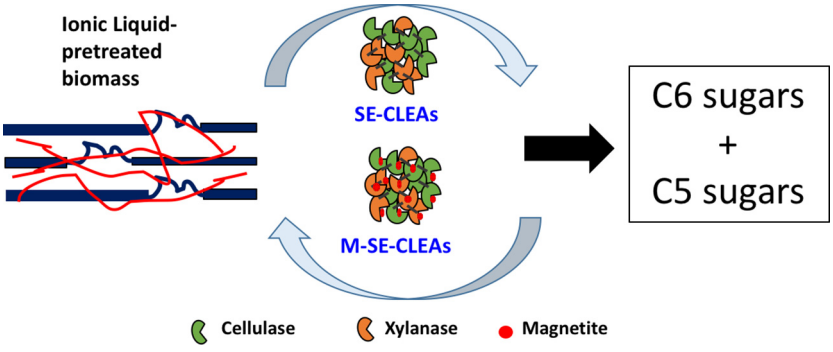
Myung Hwangbo, Janessa L. Tran, Kung-Hui Chu *

Zachry Department of Civil Engineering, Texas A&M University, College Station, TX 77843-3136, USA

HIGHLIGHTS

- Saccharifying enzymes (SEs) with similar optimal pH and temperature were produced.
- The produced SEs enabled a simple one-step enzymatic hydrolysis.
- Using the SEs resulted in 100% more sugar release than using commercial cellulases.
- Higher amounts of reduced sugar were observed using immobilized SEs than crude SEs.
- Magnetic-cross-linked enzyme aggregates allow for rapidly recover and reuse of SEs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 April 2018
Received in revised form 3 August 2018
Accepted 4 August 2018
Available online 05 August 2018

Editor: Zhen (Jason) He

Keywords:
Lignocellulosic biomass
Ionic liquid pretreatment
Saccharification
Genetic engineering
Cross-linked enzyme aggregates
Magnetic cross-linked enzyme aggregates

ABSTRACT

Lignocellulosic biomass, packed with sugars, is one of the most available renewable resources for biofuels and bioproducts production. To release the sugars for the production, enzymatic hydrolysis (saccharification) of pretreated lignocellulosic biomass are required. However, the saccharification process is costly, inefficient, and requires multi-step operations. This is in part due to the high cost and the limited selection of commercial enzymes which commonly have different optimal pH and temperatures. Here we reported a one-step saccharification of pretreated lignocellulosic biomass using immobilized biocatalysts containing five different saccharifying enzymes (SEs) with a similar optimum pH and temperature. The five SEs - endo-1,4-β-D-glucanase (an endoglucanase, eglS), cellobiohydrolase (an exoglucanase, cbhA), and β-glucosidase (bglH), endo-1,4-β-xylanase (an endoxylanase, xynC) and β-xylosidase (bxlB) – were successfully expressed and produced by *E. coli* BL21. Better saccharification of pretreated corn husks was observed when using the five crude SE enzymes than those using two commonly used SEs, endo-1,4-β-D-glucanase and β-glucosidase. The five SEs were cross-linked in the absence or the presence of magnetic nanoparticles (hereafter referred as SE-CLEAs and M-SE-CLEAs, respectively). By using SE-CLEAs, the highest amount of reduced sugar (250 mg/g biomass) was measured. The activity of immobilized SEs is better than free crude SEs. The M-SE-CLEAs can be reused at least 3 times for effective saccharification of pretreated lignocellulosic biomass.

1. Introduction

Lignocellulosic biomass, one of the most abundant renewable resources, is available for producing various biofuels and bioproducts (Billion-Ton, 2016). Lignocellulosic biomass contains 40–50% cellulose,

* Corresponding author at: 402A DLEB, Civil Engineering Department, Texas A&M University, 3136 TAMU, College Station, TX 77843-3136, USA.
E-mail address: kchu@civil.tamu.edu (K.-H. Chu).

https://doi.org/10.1016/j.scitotenv.2018.08.066
0048-9697/

25–30% hemicellulose, and 15–25% lignin (Kumar et al., 2009; Limayem and Ricke, 2012). Cellulose and hemicellulose are sugar polymers protected by a complex non-sugar, phenolic alcohol-based polymer, lignin. To extract sugars from lignocellulosic biomass for biofuels and bioproducts production, physical/chemical pretreatment followed by enzyme hydrolysis (so-called saccharification) is required (Kumar et al., 2009; Nlewem and Thrash, 2010; Wang et al., 2014). Many studies have reported that pretreatment process alone cannot achieve complete saccharification of hemicellulose (Agbor et al., 2011; Yang et al., 2011). Enzyme hydrolysis using commercial enzymes (endoglucanase and beta-glucosidase) on NaOH- or ionic liquid-pretreated biomass can release 71% of the sugars in the biomass (Xu et al., 2010). However, saccharification is costly and inefficient, in part, due to the high cost and the limited selection of commercial enzymes (Bhattacharya and Pletschke, 2014; Khare et al., 2015). The cost of commercial enzymes required for saccharification is estimated to be approximately 36% of total bioethanol production cost (i.e., $0.78 of enzyme cost to produce one gallon of ethanol from biomass) (Johnson, 2016; Lynd et al., 2008).

Cellulose is a polysaccharide consisting of repeating D-glucose (C6 sugar) with β-1,4 glycosidic bonds in a linear amorphous and/or crystalline form. Complete depolymerization of cellulose into sugar monomers requires endoglucanase to cleave amorphous cellulose, exoglucanase to cleave the crystalline cellulose, and β-glucosidase to cleave the dimers into monomer sugars (Iakiviak et al., 2016). Endoglucanase and β-glucosidase are two common commercial cellulases used in enzymatic hydrolysis for pretreated lignocellulosic biomass. Different from cellulose, hemicellulose consists various 5- and 6-carbon sugars (xylose, arabinose, galactose, mannose, glucose, and uronic acid) with β-1,4 and β-1,3 glycosidic bonds (Caes et al., 2013; Rubin, 2008) and xylan is the major component in hemicellulose (Podkaminer et al., 2012). Accordingly, different enzymes are needed to depolymerize different hemicellulose (Gupta et al., 2016). Similar to cellulose, xylan consists of repeating xylose. Depolymerization of xylan requires two types of xylanases, endoxylanase and β-xylosidase. Endoxylanase hydrolyzes (1–>4)-β-D-xylosidic linkages in xylan backbones, and β-xylosidase hydrolyzes (1–>4)-β-D-xylan units to produce xylose units by eliminating D-xylose residues (Terrasan et al., 2016). Endoxylanase produced from *Trichoderma viride* or from *Bacillus subtilis* are commercially available but their prices are much more higher than those for commercial cellulase. In addition, as these commercial cellulase and xylanase have different optimal pH and temperature, multiple steps are needed to adjust to the optimal operating conditions when using these enzymes for hydrolysis.

Cellulase and xylanase (collectively referred as saccharifying enzymes (SEs) hereafter in this study) can be produced by wild-type or genetically-modified strains (Elkins et al., 2010; Yang et al., 2011). More recently, SEs (endocellulase, β-glucosidase, endoxylanase, and xylobiosidase) have been produced by engineered *E. coli* in quantity (Bokinsky et al., 2011). However, due to the sensitivity of each of SEs to pH and temperature, there have been several trials to optimize an operating condition for saccharification (Verardi et al., 2012). Most commercial cellulase have similar optimal pH (4.0–5.0) and temperature (50–60 °C), however, commercial xylanases have various optimal conditions, with pH ranging from 4.5 to 6.0 and temperature from 30 to 50 °C. Thus, multi-step saccharification is needed when using commercial SEs for complete depolymerization of pretreated lignocellulosic biomass. A simple one-step saccharification for pretreated lignocellulosic biomass would be favorable and can be potentially achieved by using SEs that are active at pH 5.5–6.5 and 50–60 °C.

Reuse of SEs is another means to reduce the overall cost of the saccharification (Huang et al., 2015). Immobilization of enzymes not only enable reuse but also increase the activity and stability of the enzymes (Ranjbakhsh et al., 2012; Šulek et al., 2011). When commercial endoglucanase (brand name Celluclast, derived from *Trichoderma reesei*) was immobilized as cross-linked enzyme aggregates (CLEAs), the CLEAs could be reused for four times, where 40% of enzyme activity was retained at the end of the 4th cycle (Perzon et al., 2017). Previous studies have also suggested that such immobilization method would not block the active site of enzymes nor reduce the enzyme activity of SEs (Dalal et al., 2007; Sheldon, 2011). Magnetic nanoparticles (MNPs) have been recently introduced to prepare magnetic cross-linked enzyme aggregates (M-CLEAs) for easy separation between enzymes and substrates (Cui et al., 2016). M-CLEAs of bacterial xylanase (endoxylanase and β-xylosidase, respectively) from *Bacillus gelatini* ABBP-1 show 1.35-fold higher activity than the free endoxylanase or β-xylosidase (Bhattacharya and Pletschke, 2014) and M-CLEAs of lipase B can be reused up to 10 cycles by recovering easily showing a similar activity of the enzyme (Cruz-Izquierdo et al., 2014).

To address above challenges in saccharification of pretreated biomass, in this study, we reported one-step saccharification of lignocellulosic biomass using five different SEs (endoglucanase, exoglucanase, β-glucosidase, endoxylanase, and β-xylosidase) with a similar optimum pH and temperature. Xylanase were chosen in this study because xylan is a major hemicellulose. These SEs were further immobilized as reusable biocatalysts without and with MNPs (referred hereafter as SE-CLEAs and M-SE-CLEAs, respectively). SE-CLEAs, M-SE-CLEAs, and commercial enzymes were used to saccharify pretreated biomass and the effectiveness were compared based on the amount of sugars released. The reusability of SE-CLEAs and M-SE-CLEAs were also investigated.

2. Materials and methods

2.1. Chemicals

Ammonium hydroxide ($NH_3 \cdot H_2O$) was purchased from Fisher Scientific (Pittsburgh, PA). Isopropyl-β-D-1-thiogalactopyranoside (IPTG) and ammonium sulfate $(NH_3)_2SO_4$ were purchased from MP Biomedicals (Santa Ana, CA). Luria-Bertani medium and xylose were purchased from Thermo Fisher Scientific (Waltham, MA). Endo-1,4-β-D-glucanase, 0.24 U/mg from *Aspergillus niger* was purchased from TCI America (Portland, OR). β-glucosidase, 1000 U/mg from sweet almonds was purchased from MP Biomedicals (Santa Ana, CA). Xylan was purchased from Carbosynth (Berkshire, United Kingdom). All other chemicals used in this study including 1-ethyl-3-methylimidazolium chloride ([Emin][Cl]) were purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Strains and selection of genes encoding SEs

To implement one-step enzymatic hydrolysis under mild conditions, cellulases and xylanases having a similar optimum pH and temperature (pH 6.0 and temperature at 50 °C) were chosen. Based on these criteria, endo-1,4-β-D-glucanase (an endoglucanase, eglS, EC 3.2.1.6), cellobiohydrolase (an exoglucanase, cbhA, EC 3.2.1.91), and β-glucosidase (bglH, EC 3.2.1.21) for cellulase and endo-1,4-β-xylanase (an endoxylanase, xynC, EC 3.2.1.8) and β-xylosidase (bxlB, EC 3.2.1.37) for xylanase were selected. Endoglucanase (*eglS*), β-glucosidase (*bglH*), and endoxylanase (*xynC*) were derived from *Bacillus subtilis* 168 (hereafter referred as strain 168) (Bagudo et al., 2014; St John et al., 2006; Wolf et al., 1995), and exoglucanase (*cbhA*) and β-xylosidase (*bxlB*) were derived from *Aspergillus fumigatus* Af293 (hereafter referred as strain Af293) (Adav et al., 2013; Grajek, 1986). Table 1 summarizes the information of the genes encoding these enzymes.

2.3. Plasmid construction for SEs expression

The genes encoding the SEs described above were used to construct recombinant plasmids using In-Fusion® HD Cloning Kit (Clontech, Mountain View, CA) (Fig. 1a). The genomic DNA of strains 168 was extracted using FastDNA SPIN KIT (MP Biomedicals, Santa Ana, CA) after growing in LB medium at 37 °C for overnight, and cDNA of strains Af293 was directly used for PCR amplification. The PCR primers were designed based on the sequence of strains 168 and Af293, and each gene was amplified using 10 primers as listed in Table 2. A 25-μL of