



Effect of pretreatment methods on the synergism of cellulase and xylanase during the hydrolysis of bagasse



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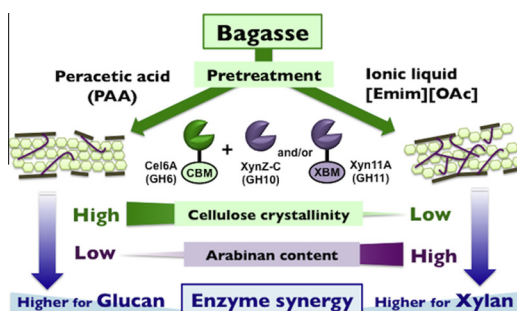
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HIGHLIGHTS

- Pretreatments resulted in different compositional and structural changes on bagasse.
- Presence of arabinan inhibited xylanase action on pretreated bagasse.
- Cellulose crystallinity and hemicellulose structure impacted on enzyme synergism.
- Variation of enzyme molecular structure affected the synergistic action.

GRAPHICAL ABSTRACT



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ABSTRACT

The effect of pretreatment with peracetic acid (PAA) or an ionic liquid (1-ethyl-3-methylimidazolium acetate, [Emim][OAc]) on the synergism between endoglucanase and endoxylanase in the hydrolysis of bagasse was investigated. An endoglucanase, Cel6A, with a carbohydrate-binding module (CBM) and two endoxylanases, XynZ-C without a CBM and Xyn11A with an intrinsic xylan/cellulose binding module (XBM), were selected. The hemicellulose content, especially arabinan, and the cellulose crystallinity of bagasse were found to affect the cellulase–xylanase synergism. More specifically, higher synergism (above 3.4) was observed for glucan conversion, at low levels of arabinan (0.9%), during the hydrolysis of PAA pretreated bagasse. In contrast, [Emim][OAc] pretreated bagasse, showed lower cellulose crystallinity and achieved higher synergism (over 1.9) for xylan conversion. Ultimately, the combination of Cel6A and Xyn11A resulted in higher synergism for glucan conversion than the combination of Cel6A with XynZ-C, indicating the importance of the molecular architecture of enzymes for metabolic synergism.

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1. Introduction

Second-generation bioethanol, produced from agriculture residues, as a substitute for fossil fuels has received widespread attention since the end of the last century. Owing to the prior abundance and appreciable polysaccharides percentage, sugarcane bagasse is considered an ideal substrate for biorefineries (Chandel et al.,

2012). However, because of the complex matrix of polysaccharides and lignin within the structure of the plant cell wall, pretreatment becomes an indispensable step to accelerate the degradation of the lignin–carbohydrate complex and to ensure that bioethanol production is a competitive and sustainable process (Davis et al., 2013).

Studies aimed at improving monosaccharide production from sugarcane bagasse include the use of acid, base, hydrogen peroxide and steam explosive processes (Lv et al., 2013; Sant'Ana da Silva et al., 2011). Previous studies showed that peracetic acid (PAA) has good delignification ability, because PAA generates the hydroxonium ion, HO^+ , which attacks the electron sites of lignin related structure via an electrophilic reaction (Kumar et al., 2013; Uju et al., 2013; Zhao et al., 2007). Ionic liquids (IL) as emerging reagents for biomass pretreatment have been extensively studied because of their excellent characteristics and unique cellulose dissolution capability (Brandt et al., 2013; Mora-Pale et al., 2011). This dissolution is induced by the formation of the electron donor–acceptor complex between the anion of IL and the free hydroxyl group on cellulose, and between the cation of IL and the hydroxyl oxygen atoms on the cellulose chain. Among the different ILs, [Emim][OAc] has proven to be one of the most effective in solubilizing cellulose (Brandt et al., 2013).

Naturally, xylans are highly cross-linked with cellulose fibrils by diferulic bridges, contributing to the firm cell wall matrix (Saha, 2003). As a result, access to cellulose by cellulase is generally prevented and this reduction in cellulose activity can hamper downstream successive enzymatic hydrolysis processes. Nonetheless, hemicellulose is a desired polysaccharide that can also be yielded from biomass conversion. The addition of accessory enzymes, especially xylanases, to degrade the hemicellulose content is an alternative approach to make cellulose more accessible to cellulases, and to yield more saccharides simultaneously. Thus, the synergistic use of xylanases with cellulases has become an attractive approach for cellulose degradation. Research using commercial enzymes, which contain a consortium of enzymes, have demonstrated the synergism between cellulases and xylanases (Kumar and Wyman, 2009a; Li et al., 2014; Zhang and Viikari, 2014). In contrast, there are specific efforts that have focused on the synergism between pure enzyme components. Selig and co-workers have demonstrated a significant increase in the depolymerization of hot water treated corn stover by cellobiohydrolase I (CBHI) with the addition of endoxylanase and/or other accessory enzymes (ferulic acid esterase, acetyl xylan esterase) (Selig et al., 2008). A recent report has shown the synergy between CBHI (Cel7A), endoxylanases (GH10 and GH11) and/or xyloglucanase (GH5) during hydrolysis of steam pretreated lignocellulosic biomass substrates; however, the reaction efficiency was highly substrate specific (Hu et al., 2013).

Previous work has shown the use of bagasse as a substrate to study the synergistic effect between cellulase and xylanase. High synergy (6.3) between endoxylanase Xyn11A from *Cellulomonas flavigena* and endoglucanase Cel7B from *Trichoderma reesei* for reducing sugars production has been reported during the hydrolysis of alkaline pretreated bagasse (Pavón-Orozco et al., 2012). Nonetheless, a recent report using four types of reagents to pretreat bagasse observed distinct synergism between cellulase and xylanase. Steam exploded bagasse, NaOH and H_2O_2 pretreated bagasse showed obvious synergy between cellulase and xylanase for glucose and xylose production, whereas H_2SO_4 pretreated bagasse did not (Li et al., 2014). Thus, it appeared that synergism between cellulase and xylanase was specific to the substrate and the pretreatment method used.

In the present work, the physical structure and chemical composition of bagasse after PAA and [Emim][OAc] pretreatments were

determined to verify the potential synergistic effect between endoglucanase and endoxylanase. For rationalizing the action of the hydrolases with different catalytic properties, one endoglucanase, Cel6A (GH6, with a CBM) from *Thermobifida fusca*, and two different endoxylanases, XynZ-C (GH10, without a CBM) from *Clostridium thermocellum* and Xyn11A (GH11, with a XBM) from *T. fusca*, were selected. The performance of each enzyme with varied molecular structure in the hydrolysis of pretreated bagasse with different chemical and physical properties was systematically investigated.

2. Methods

2.1. Materials and enzymes

The biomass bagasse with an average particle size of 200 μm was purchased from Toyota Tsusho Corporation (Nagoya, Japan). 1-Ethyl-3-methylimidazolium acetate ([Emim][OAc]) was purchased from Kanto Kagaku, Tokyo, Japan. PAA was supplied by the Mitsubishi Gas Chemical Company, Inc., Japan. The details of the expression and purification of enzymes used in this study are provided in the Supplementary information. The enzyme purities were confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Fig. S1).

2.2. Biomass pretreatments and regeneration

Fifty milligrams of bagasse was added to 1 g [Emim][OAc] or 1 mL deionized water that includes 50 μL of a 40% (w/w) PAA solution. The mixture was heated at 80 °C with stirring (200 rpm) for 3 h. After the reaction, 5 mL deionized water was added into the tubes to precipitate the regenerated cellulose with vigorous shaking. Subsequently, the regenerated bagasse and solution were separated by centrifugation at 25 °C (5800g, 20 min). The regenerated water was kept at –18 °C for further investigation. In order to remove the residual reagent, the regenerated cellulose was washed with 5 mL deionized water three times. The regenerated cellulose was then dried by lyophilization overnight. All pretreatment experiments were carried out in triplicates. The dry mass of the regenerated cellulose was weighed and the yield of the regenerated biomass was calculated by the following equation (Uju et al., 2013):

$$\text{YRB (\%)} = M_{\text{pretreated biomass}} / M_{\text{initial biomass}} \times 100\% \quad (1)$$

2.3. Chemical composition of bagasse and sugar loss in regenerated water during the pretreatment process

The chemical composition of untreated, PAA pretreated and [Emim][OAc] pretreated bagasse was measured following the protocol (LAP TP-510-42619, 42618, 42622) from the National Renewable Energy Laboratory (Sluiter et al., 2011, 2008a,b). Measuring sugar loss in regenerated water was performed with small modifications. Briefly, the samples were treated with 72% sulfuric acid at 30 °C for 1 h, then diluted to 4% and incubated in an autoclave at 121 °C for 1 h. After cooling the sample, calcium carbonate was used for neutralizing the pH to 7. Analysis of the sugar content was performed using a high-performance liquid chromatography (HPLC) system equipped with a Shodex sugar KS-801 column (8.0 $\times$ 300 mm, Showa Denko Co., Tokyo, Japan) and an RI detector at 80 °C with HPLC-grade water as the eluent at a flow rate of 1 mL/min. The remaining acid insoluble lignin was measured after oven-drying overnight at 45 °C and the amount of acid soluble lignin was determined by measuring the

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