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Optimization of high solids fed-batch saccharification of sugarcane bagasse based on system viscosity changes

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ABSTRACT

Viscosity trends in alkali-pretreated sugarcane bagasse (SCB) slurries undergoing high solids fed-batch enzymatic hydrolysis were measured for a range of solids loading from 15% to 36%. Solids liquefaction times were related to system viscosity changes. The viscosity decreased quickly for low solids loading, and increased with increasing solids content. Fed-batch hydrolysis was initiated with 15% solids loading, and an additional 8%, 7% and 6% were successively added after the system viscosity decreased to stable values to achieve a final solids content of 36%. Two enzyme-adding modes with 8.5 FPU/g solid were investigated. The batch mode with all enzyme being added at the beginning of the reaction produced the highest yields, with approximately 231.7 g/L total sugars and 134.9 g/L glucose being obtained after 96 h with nearly 60% of the final glucan conversion rate. This finding indicates that under the right conditions, the fed-batch strategy might be a plausible way to produce high sugars under high solids.

1. Introduction

Enzymatic hydrolysis of lignocelluloses has long been investigated to depolymerize biomass into fermentable sugars for biofuel production, with a more recent focus on operating at high-solids loading. A process is considered to be high-solids when the ratio of solids/liquid is very high and no free water is present, or roughly a solid loading above 15% (w/v). High-solids saccharification, with lower capital costs due to fewer reactors, less energy consumption for heating and cooling, and reduced disposal costs due to less water usage, is a direct and convenient technique to obtain high sugars (Jørgensen et al., 2007; Kristensen et al., 2009; Zhang et al., 2013).

However, challenges such as increased viscosity resulting in mass transfer limitations, stirring difficulties, and inhibition from toxic products such as fermentation inhibitors and lignin are common to operations at high-solids loading (Alvira et al., 2013; Jørgensen et al., 2007; Roche et al., 2009b). Additionally, a high enzyme dosage is required under these conditions (Zhang et al., 2006). To overcome these technical barriers, novel strategies have been demonstrated (Lan et al., 2013; Hoyer et al., 2013). Fed-batch schemes, as an alternative method of achieving high-solids loading has been investigated for its various advantages (Hodge et al., 2009; Rosgaard et al., 2007). Its feeding regime allows time for the solids to liquefy before adding additional substrate. The system diffusion and mixing limitations can be minimized because its initial viscosity is lower. Zhang et al. (2012) studied the fed-batch approach with different solids additions over the course of 48 h to achieve a substrate loading of 30% (w/v), and a final glucose conversion of 55%. Ma et al. (2011) investigated 25% solids loading with the enzymes added either all at once at the beginning of the reaction or with each addition of the acid-pretreated cassava bagasse, and the fed-batch with a single enzyme addition achieved ~75% conversion. These studies obtained the desired high conversion rate by applying fed-batch systems under relatively right conditions, but the viscosity of the systems as an evaluation index all were not investigated. At higher solids loading, the apparent viscosity and yield stress increased with the volume fraction of insoluble solids, which made the rheology of the biomass slurry markedly non-Newtonian. Here, the yield stress is the stress at the viscosity maximum that must be applied to a sample before it starts to flow. Normally the continuous phase is low in viscosity, but high concentrations of a dispersed phase and/or strong interactions between components can increase the viscosity. (Larson, 1999; Ehrhardt, 2008; Stickel et al., 2009; Samaniuk et al., 2012). Fundamental understanding of the rheol-

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ogy of these slurries is a powerful tool in designing conversion processes and equipment (Knutsen and Liberatore, 2009).

Enzyme hydrolysis can alter the rheological properties of biomass. Ehrhardt et al. (2010) measured the flow resistance of acid-pretreated corn stalks with a torque rheometer, and found that it decreased with time after enzyme addition. Roche and Dibble, 2009a found that the yield stress of the same substrate decreased with time in enzymatic hydrolysis.

Although many advantages have been realized with the use of fed-batch systems, its feeding schemes are currently still inconclusive. When this approach was selected, how and when to add substrates and enzymes to the reaction to maintain a relatively low viscosity throughout hydrolysis and achieve a high conversion rate should be considered. In the present study, alkali-pretreated sugarcane bagasse (SCB) with a high cellulose content was chosen as a substrate to obtain high sugars with the aim of looking for a cost-effective ethanol production approach, and the optimal fed-batch feeding condition and enzyme addition mode with the variation of viscosity during enzymatic hydrolysis were investigated at a low enzyme loading of 8.5 FPU/g substrate.

2. Materials and methods

2.1. Materials

SCB was obtained from Guangxi Fenghao Sugar Co., Ltd (Chongzuo, China). It was milled and screened, and the fraction between 20 and 40 meshes with approximately 0.5 to 1.0 cm length was used for alkali-pretreatment. A cellulase mixture, Cellic CTec2, was kindly provided by Novozymes A/S (Bagsaevrd, Denmark), with an enzyme activity of approximately 200 FPU/mL, as measured by the description of IUPAC (Ghose, 1987). All other chemicals were of analytical grade.

2.2. Alkali pretreatment

Details of the dilute and low-temperature alkali-pretreatment have been described elsewhere (Zhang et al., 2012). 1 g of dry SCB was mixed with 20 mL 0.5 M NaOH solution at a solid/liquid ratio of 1:20 in a round-bottom flask at 80 °C for 2 h with agitation. After reaction, the pretreated materials were washed with tap water until the washing liquid was neutral. The obtained residues were oven-dried at 50 °C for 24 h. The pretreated redidues became fractured and showed a rough structure with obvious cracks, ravines and holes, as a result of lignin removal. These changes were conducive to the subsequent enzymatic hydrolysis. All experiments were carried out three times, and the given numbers are the mean values.

2.3. Enzymatic hydrolysis

The pretreated substrate was dried and storage in a clean container. The batch enzymatic hydrolysis was carried out with solids loading of 9%, 12%, 15% and 18% dry mass (DM) (w/v) (the actual amounts were 9, 12, 15, and 18 g, respectively) at 50 °C and 150 rpm in 250 mL Erlenmeyer flasks and sealed with rubber stoppers, each containing 100 mL of 0.05 M acetate buffer (pH 5.0). For the hydrolytic reactions, the enzyme loading was 8.5 FPU/g substrate (43 mg/g substrate), and dosing was based on the final total amount of DM loaded into the reaction. After sampling, the enzyme was inactivated (boiling at 100 °C for 5 min) and the released sugars were analyzed by HPLC.

High-solids fed-batch enzymatic hydrolysis was initiated with 15% solids loading, which was optimized from the batch process, and then the system viscosity measurement was simultaneously started at specific time points during the hydrolysis. After the viscosity was assumed to reach a steady-state value, i.e., the viscosity value reading was reduced below the maximum measuring range of the viscometer, new substrate was quickly added to avoid contamination. The substrate addition time was determined by the system viscosity change, and 8% (w/v), 7% (w/v), and 6% (w/v) substrate were fed consecutively when the viscosity dropped below the shear rates accessible with the torque rheometer, respectively. The letters w and v represented the solid mass (g) and the liquid volume (mL), respectively. Enzyme was added along with the feeding amount of the substrates, or it was added all at once at the beginning of the reaction according to the final solids concentration.

Only pure and dried solid was fed at each time point, so the final solid and enzyme loading were 36% and 8.5 FPU/g substrate, respectively. All the reactions were performed in 250 mL Erlenmeyer flasks sealed with rubber stoppers, at 50 °C, 150 rpm, and pH 5.0 (0.2 M acetate buffer). At each desired time, the solution was sampled for sugars assay.

2.4. Analytical methods

2.4.1. Sugar assay

The dissolved sugar content was determined by a Waters 2695 high-performance liquid chromatography (HPLC) system using a Shodex sugar SH-1011 column coupled with a refractive index detector. The mobile phase of 5 mM H_2SO_4 in demineralized water was used at a flow rate of 0.5 mL/min at 50 °C. Standard and hydrolyzed samples were filtrated by a 0.22 μm filter before analysis.

2.4.2. Composition assay

The components of the SCB before and after the pretreatment were analyzed according to the standardized methods of the National Renewable Energy Laboratory (NREL, Golden, CO, USA) (Sluiter et al., 2008).

2.4.3. Viscosity measurements

The apparent viscosities of the slurries were measured every hour at the beginning of the hydrolysis using a DV Ultra rheometer operating in controlled shear rate mode and equipped with a coaxial cylinder measurement geometry with a diameter of 2.1 cm. Samples for the rheology tests were subjected to mixing over a period of 45 s to capture the non-thixotropic flow behavior of the slurry under a rotor speed of 250 rpm. Each test was repeated between 8 and 10 times with different samples to ensure the reproducibility of the data. Accurate measurements of the viscosity require a period of premixing. To determine the time required to reach steady-state for high-solids systems, the viscosity was measured as a function of time at 250 rpm. The time to reach steady-state varied with the initial solids concentration, the 250 rpm corresponding to an impeller speed that is 30% higher than that necessary to suspend all particles, as estimated for the suspension of a solid substrate in a liquid on the lab scale.

3. Results and discussion

3.1. Substrate composition

Lignin, with its protective sheathing, is considered to impede enzymes' access to glucan chains and reduce the cellulase efficiency due to unproductive binding and steric hindrance (Chang and Holtzapple, 2000). Its removal is an effective way to increase the enzymatic hydrolysis efficiency and sugar yield (Balat et al., 2008). To improve enzymes access, alkali-pretreatment was carried out. As shown in Table 1, the untreated SCB was composed of glucan, xylan, and lignin in percentages of 38.6%, 23.6% and 21.2%,