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Enzymatic Hydrolysis with Cellic CTec3 at High Total Solids and Cellulosic Ethanol Production

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ABSTRACT

Cellulosic ethanol is one of the most important biotechnological products to mitigate the consumption of fossil fuels and to increase the use of renewable sources for fuels and chemicals. In this work, steam-exploded sugarcane bagasse was used for enzymatic hydrolysis at high substrate total solids (TS). Different factorial designs were carried out by varying TS and enzyme loading using Cellic CTec3 and Cellic HTec3. Hydrolyses were performed in shake flasks to select the best conditions for a subsequent scale-up to a 3.2 L reactor. The best hydrolysis conditions were at 20 wt.% TS and 22.1 FPU g^{-1} TS, reaching 98 g L^{-1} glucose in 72 h. In general, Cellic CTec3 reduced the substrate apparent viscosity very quickly and Cellic HTec3 did not improve glucan conversion. Hydrolysis at 20 wt.% in larger scale reached 103 g L^{-1} glucose in 72 h using only 13.2 FPU g^{-1} TS of Cellic CTec3.

Keywords: sugarcane bagasse, apparent viscosity, cellulosic ethanol, Cellic CTec3, Cellic HTec3, Thermosacc Dry.

INTRODUCTION

Cellulosic ethanol is produced by fermentation of carbohydrates that are released from plant polysaccharides by enzymatic hydrolysis. Such production process depends on five sequential steps involving: (1) collection and preparation of plant biomass; (2) pre-treatment, which aims to increase the accessibility of plant polysaccharides to bioconversion at high process yields; (3) enzymatic hydrolysis, which converts plant polysaccharides into fermentable sugars; (4) microbial fermentation, which is responsible for the production of ethanol; and finally, (5) the recovery of the ethanol by distillation (Cara et al., 2008).

Steam explosion is one of the most widely used methods for pretreatment (Ramos, 2003; Carrasco et al., 2010). This method increases the accessibility of cellulose by deconstructing the associative structure of plant cell wall, causing an increase the substrate surface area and pore volume. Changes are also introduced in the biomass chemistry, particularly by the partial acid hydrolysis of hemicelluloses and lignin (Schütt et al., 2012).

Enzymatic hydrolysis is accomplished by different classes of enzymes which are intended to convert all of the available plant polysaccharides into fermentable sugars. Performing this step in high consistency, using high total solids and relatively low enzyme loadings, can offer significant advantages to the cellulosic ethanol production process. According to Ramos et al. (2014), these advantages involve the reduction in capital cost for hydrolysis and distillation, particularly due to the fact that high sugar concentrations are produced in the substrate hydrolysate for fermentation. The main goal of this work was to

produce high concentrations of fermentable sugar from steam-exploded sugarcane bagasse and to evaluate the effect of hydrolysis on the substrate apparent viscosity.

METHODOLOGY

2.1. Pretreatment and chemical composition of lignocellulosic materials

Sugarcane bagasse was provided by Cane Technology Center (CTC - Piracicaba, SP, Brazil). Pretreatment was carried out by autohydrolysis at 195 °C for 7.5 min under conditions that were pre-optimized recently (Pitarelo et al, 2016). Water washing was applied on the resulting pretreated materials to remove water-soluble hemicellulose and lignin components. The composition analysis of untreated and steam-treated materials was carried out according to NREL methods. The carbohydrates were analyzed by high performance liquid chromatography (HPLC) after dilute sulfuric acid hydrolysis.

2.2. Enzymatic hydrolysis and rheological analysis

Pretreated sugarcane bagasse was hydrolyzed with Cellic CTec3 (Novozymes Latin America - Araucaria, PR, Brazil) using a 2^2 factorial design with a star configuration (four axial points) and three center points as shown in the **Table 1**. The apparent viscosity of these substrates were obtained in flow experiments before and during enzymatic hydrolysis using a controlled-stress rheometer (TA instruments, New Castle, DE, USA) with vane-in-cup geometries. After selecting the best hydrolysis condition, another 2^2 factorial design was performed at 20 wt.% TS varying both Cellic CTec3 (4.4 and 22.1 FPU g^{-1} TS) and Cellic HTec3 (0 and 10 % in relation to CTec3) concentrations. All experiments were carried out in shake flasks using acetate buffer 50 mol L^{-1} pH 5.2 at 50 °C for 72 h and 150 rpm. Sample aliquots were collected at 3, 6, 9, 12, 24, 48, and 72 h and analyzed by HPLC.

2.3. Scale-up enzymatic hydrolysis and fermentation

Enzymatic hydrolysis was performed in 3.2 L Labfors 5 BioEtOH bioreactor (Infors-HT) with multiple stirrers during 72 h at 50 °C and 150 rpm, using 13.2 FPU of Cellic CTec3 g^{-1} TS in 50 mol L^{-1} acetate buffer pH 5.2. The reactor feeding was done by fed-batch with 5 wt.% TS of pretreated material being added at every 1.5 h until 20 wt.% was reached.

The fermentation medium contained 20 mL of hydrolysate, 50 mmol L^{-1} acetate buffer pH 4.8, 1.0 g L^{-1} yeast extract, 0.5 g L^{-1} $(NH_4)_2PO_4$, 0.025 g L^{-1} $MgSO_4.7H_2O$ and 1.0 g L^{-1} of *Saccharomyces cerevisiae* Thermosacc Dry (Lallemand - Milwaukee, WI, USA). Reactions were carried out by 20 h at 35 °C in shake flasks. Aliquots of 0.2 mL were withdrawn in different times and these were also analysed by HPLC.

RESULTS AND DISCUSSION

3.1. Chemical analysis and pre-treatment by steam explosion

Sugarcane bagasse contained 34.6 % of glucans and 16.8 % of xylans. The mass recovery yield after pretreatment was 64 wt.% and water-washed steam-exploded bagasse contained 54.7 % and 3.4 % of glucans and xylans, respectively.

3.2. Enzymatic hydrolysis and rheology analysis

The largest glucan conversion after 72 h of hydrolysis were obtained from conditions

#7, #9 and #10 of **Table 1**, and these were equivalent to 57.2, 84.2 and 46.2 g L^{-1} glucose, respectively. These experiments also showed the fastest initial hydrolysis rates, which were followed by the lowest apparent viscosity values (at 10 s^{-1}) as observed in **Table 1**. In general, higher viscosities were obtained at lower glucan conversions. The apparent viscosity at the reaction beginning was 129.7, 93.1, 53.5, 23.4 and 15.4 Pa s in average for experiments at 22, 20, 15, 10 and 8 wt.% TS, respectively. However, in the first 3 h of hydrolysis, there was a subtle decrease in the apparent viscosities, mainly for situations in which the highest enzyme loadings were used, being 81% lower for condition #2, 97 % lower for condition #7 and 89 % lower for condition #9.

Table 1. Experimental conditions, glucan conversion (%) and apparent viscosity in 10 s^{-1} for the enzymatic hydrolysis of steam-treated sugarcane bagasse.

Run	EL[a] (FPU)	TS[b] (wt.%)	Glucan Conversion (%)			Apparent viscosity (Pa s)		
			3 h	12 h	24 h	3 h	12 h	24 h
1	4.4	20	15.6	27.0	38.6	39.3	17.6	9.7
2	22.1	20	20.8	43.5	59.9	18.1	1.8	0.4
3	13.2	15	16.6	44.7	60.7	17.2	1.1	0.2
4	13.2	15	19.9	42.4	60.0	15.3	0.9	0.2
5	13.2	15	16.2	44.8	60.4	14.5	0.9	0.2
6	4.4	10	17.0	37.4	54.5	16.8	1.1	0.3
7	22.1	10	43.4	73.6	88.4	0.7	0.04	0.01
8	0.8	15	2.8	6.6	11.2	44.2	23.4	15.2
9	25.7	15	30.6	60.6	82.6	6.0	0.3	0.06
10	13.2	8	33.0	61.8	80.4	4.4	0.1	0.02
11	13.2	22	18.9	38.5	49.6	20.3	4.5	2.8

[a] Enzyme Loading: FPU per gram of total solids; [b] Total Solids.

The highest glucose concentrations at 12 h were achieved for conditions #2 and #9, which were equivalent to glucose concentration of around 52 g L^{-1}. On the other hand, condition #11 resulted in a similar glucose release but for a much lower conversion value and a higher apparent viscosity (**Table 1**). Therefore, condition #2 was the best among those tested in this study because the lowest viscosity value was reached in 24 h for a total glucose release of 70 g L^{-1}. Also, after 48 h of hydrolysis, this same experiment reached 87 g L^{-1} glucose, which is already enough to make fermentation economically viable.

In an attempt to improve the enzymatic hydrolysis even further, a new set of experiments based on condition #2 was organized in a typical 2^2 factorial design to evaluate the effect of Cellic HTec3 hemicellulases. The results indicated that Cellic HTec3 caused only a slight increase in glucan conversion. Also, by comparing hydrolysis experiments that used 22.1 FPU g^{-1} TS with those of the center point (13.2 FPU g^{-1} TS and 5% HTec3), an increase of only 9 % was obtained in glucose concentration. Thus, the enzyme loading of 13.2 FPU g^{-1} TS was used for the subsequent scale-up experiments.

3.3 Scale-up enzymatic hydrolysis and fermentation

The scale-up enzymatic hydrolysis experiments reached 103 g L^{-1} glucose in 72 h for a glucan conversion of 95 %. With regard to fermentation, substrate hydrolysates were readily

fermented in good yields by an industrial yeast strain of *S. cerevisiae*, achieving ethanol productivity values of 2.2 g L^{-1} h^{-1} after only 12 h and ethanol yields higher than 87 %.

Sugarcane reaches harvest maturity after 12 months in equatorial and hot tropical regions (Ramburan, 2015) and the average annual productivity is around 20.16 t ha^{-1} $year^{-1}$ (Carvalho et al., 2015). Hence, when these results are projected to 1 t of processed biomass, sugarcane bagasse plantations are able to achieve cellulosic ethanol productivities of 1794.5 L ha^{-1} $year^{-1}$. Sugarcane has the advantage of producing ethanol from first generation, which is estimated to be 80 L t^{-1} in average (Leal and Nogueira, 2014). Based on the average biomass productivity of 72 t ha^{-1} $year^{-1}$ that was obtained in the last Brazilian harvest season (Carvalho et al., 2015), this corresponds to 5760.0 L ha^{-1} $year^{-1}$ of ethanol on wet basis or a total of 7554.5 L ha^{-1} $year^{-1}$ if both first and second generation technologies are added together.

CONCLUSION

Hydrolysis at 20 wt.% TS with 22.1 FPU g^{-1} TS resulted in 98 g L^{-1} glucose in 72 h. In general, Cellic CTec3 reduced the substrate apparent viscosity very quickly, except for experiments in which low enzyme loadings were employed. The presence of Cellic HTec3 had no influence on the glucan conversion at high TS. The scale-up hydrolysis experiments reached 103 g L^{-1} glucose in 72 h using only 13.2 FPU g^{-1} TS. When this result was projected to 1 t of processed biomass, sugarcane bagasse glucans showed the potential to boost the ethanol production from sugarcane culms by 31 %, from the 80 L t^{-1} of first generation to a total production of 105 L t^{-1}. This number was obtained without considering the yield increments that are expected to arise from the C5 stream as well as from the utilization of other harvest residues for the same purpose, such as sugarcane leaves and tops.

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