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Assessment of different biomass feeding strategies for improving the enzymatic hydrolysis of sugarcane straw

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ABSTRACT

This work describes improvements in the enzymatic hydrolysis of hydrothermally pretreated sugarcane straw. The experiments were performed in batch and fed-batch operation modes (50 mL and 3 L working volumes) with final solid loadings of 10, 20, and 30% (w/v) and enzyme dosage of 10 FPU/$g_{substrate}$. The fed-batch assays showed the most promising results in terms of glucose production and cellulose-to-glucose conversion. In the experiments using a 3 L working volume, analysis of cellulose-to-glucose conversion, power consumption, and apparent viscosity showed that the best assays were those with smoother feed delivery (substrate plus enzyme) throughout the enzymatic hydrolysis. This strategy resulted in 108.8 g/L of glucose and cellulose-to-glucose conversion of 59.7%, corresponding to a final theoretical ethanol content of 55.6 g/L. The power consumption per unit volume and the apparent viscosity were less than 5 kW/m^3 and 35 mPa s, respectively, throughout the hydrolysis. Thus, a more concentrated liquor and a less powerful motor to deliver power during the hydrolysis process were obtained in fed-batch strategy reducing the process energy costs.

1. Introduction

In production of bioethanol from lignocellulosic biomass, the use of a high solid loadings for enzymatic hydrolysis, such as 20–25% water insoluble solids (Cardona et al., 2014; Du et al., 2014; Huang et al., 2013), can provide a high sugar concentration in the hydrolysis liquor and therefore increase the energy efficiency of the distillation step (Fujii et al., 2014; Ximenes et al., 2010). On the other hand, when high lignocellulosic solids content is employed, the conversion of carbohydrates to sugar monomers may be restricted by mixing difficulties and the inhibition/deactivation of enzymes (Cardona et al., 2014; Fujii et al., 2014; Xue et al., 2012; Ximenes et al., 2011). Several studies have investigated this issue (Santos-Rocha et al., 2017; Corrêa et al., 2016a; Kadić and Lidén, 2017; Pratto et al., 2016; Sanchez and Gomez, 2014) but improving the energy efficiency of large-scale second generation ethanol production remains a major challenge.

During the enzymatic hydrolysis step, high concentrations of water insoluble solids can cause both mass and heat transfer problems, due to poor mixing of the material. Satisfactory homogeneity of the suspension requires high energy input during the production of ethanol from biomass, and difficulties arise in the design of suitable reactors able to operate with maximum efficiency and minimum power consumption (Palmqvist et al., 2011; Cardona Alzate and Sánchez Toro, 2006). Fan et al. (2003) performed enzymatic hydrolysis experiments of paper sludge in a semi-continuous solids-fed reactor using operating conditions similar to those expected in an industrial process. The authors verified that the power requirements for mixing the slurry during the hydrolysis sharply increased as the solids concentration increased, indicating that this property is notably important to be considered during studies of high solid loadings enzymatic hydrolysis. Since the rheology of the medium is strongly dependent on the water insoluble solids concentration, the challenge associated with processing a high biomass load in the enzymatic hydrolysis reactor is to find the optimal conditions that result in high sugar conversion, low viscosity, and low power consumption (Hou et al., 2016; Pereira et al., 2011). Rheology is a branch of science, especially mechanics, which studies the deformations

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Table 1
Substrate and enzyme feeding strategies for sugarcane straw enzymatic hydrolysis.

50 mL working volume	Batch strategies	Solids loadings (%, w/v) and enzyme (E)	Feed time (h)
	S1	10 (E)	0
	S2	20 (E)	0
	S3	30 (E)	0
	Fed-batch strategies	Solids loadings (%, w/v) and enzyme (E)	Feed time (h)
	S4	5(E) + 5(E)	0, 24
	S5	5(E) + 5(E) + 5(E) + 5(E)	0, 2, 12, 24
	S6	5(E) + 5(E) + 5(E) + 5(E) + 5(E) + 5(E)	0, 2, 4, 8, 12, 24
3 L working volume	Fed-batch strategies	Solids loadings (%, w/v) and enzyme (E)	Feed time (h)
	S7	5(E) + 5(E) + 5(E) + 5(E) + 5(E) + 5(E)	0, 2, 4, 8, 24, 30
	S8	5(E) + 5(E) + 5(E) + 5(E) + 5(E) + 5(E)	0, 2, 12, 24, 36, 48
	S9	2.5(E) + 2.5(E) + 2.5(E) + 2.5(E) + 2.5(E) + 2.5(E) + + 2.5(E) + 2.5(E) + 2.5(E) + 2.5(E) + 2.5(E) + 2.5(E)	0, 1, 2, 4, 8, 12, 18, 24, 30, 36, 42, 48

Enzyme dosage was fixed in 10 FPU/$g_{substrate}$ for all evaluated batch and fed-batch strategies. In fed-batch strategies the substrate (%, w/v) and enzyme dosage (E) were divided and fed into the reactor at different hydrolysis time.

and flows of matter under stress, strain, temperature, humidity, and other conditions. During the enzymatic hydrolysis reaction, analysis of rheological properties, especially yield stress, becomes very helpful for the reactor design and the process optimization aiming scale-up estimates. In this way, an understanding of how the rheological behavior of biomass slurries changes during saccharification step is of paramount importance (Chen and Liu, 2017; Knutsen and Liberatore, 2009; Roche et al., 2009).

The fed-batch operational mode is promising for enzymatic hydrolyses at high solid loadings, with acceptable power consumption, rheological behavior, and cellulose-to-glucose conversion. This strategy can allow use of ideal blends when large amounts of dry matter are employed in the hydrolyses (Corrêa et al., 2016b; Sotaniemi et al., 2016; Cavalcanti-Montaño et al., 2013). The literature reports that fed-batch strategy schemes presents lower initial viscosity compared to batch process operation. Fed-batch operation provides timely manner for the biomass becomes liquefied before adding additional solids while maintaining considerable amount of free water in the initial stages of the enzymatic hydrolysis, which minimizes or altogether avoids mixing difficulties. Additionally, fed-batch process can be helpful for the reduction of enzymes inhibition effect (Chen and Liu, 2017; Modenbach and Nokes, 2013).

Various lignocellulosic substrates have been employed in studies of enzymatic hydrolysis, including corn stover, hardwood chips, wheat straw, spruce, and sugarcane bagasse (Kadić and Lidén, 2017; Kumar et al., 2015; Du et al., 2014; Pihlajaniemi et al., 2014; Xue et al., 2012). Several factors including inhibition effects, water constraint, and rheology characteristic in enzymatic hydrolysis of lignocellulosic biomass were addressed in terms of both challenges and perspectives in a recent review by Chen and Liu (2017). The authors point out that fed-batch process and different feeding strategies should be fully exploited in order to overcome process limitation of the hydrolysis step. To the best of our knowledge, there have been no systematic studies of 2 G ethanol production that have considered the power consumption and rheological behavior during enzymatic hydrolysis of hydrothermally pretreated sugarcane straw, at high solid loadings. The present work is intended to fill this gap in knowledge.

Therefore, this study presents a proposal for improvements in the enzymatic hydrolysis of hydrothermally pretreated sugarcane straw, using batch and fed-batch operating modes, at solid loadings of 10, 20, and 30% (w/v). Experiments were conducted to find the best strategy for substrate feeding during enzymatic hydrolysis in a 3 L (3 dm^3) working volume reactor, at a solid loading of 30% (w/v).

The rheological, power consumption, and process conversion data obtained enabled proposal of a suitable feeding strategy to be applied in stirred bioreactors for scaled-up sugarcane straw enzymatic hydrolysis. The fed-batch approach can assist in making the hydrolysis step economically feasible, and recent developments in bioreactor engineering have focused on this type of operational strategy.

2. Materials and methods

2.1. Materials

The sugarcane straw (SCS) used in the hydrolysis experiments was kindly donated by Ipiranga Agroindustrial S. A. Mill (Descalvado, São Paulo, Brazil). The ground SCS was dried at room temperature until it was approximately 10% in moisture content. After, it was milled in a Willey type mill (model SP-30, SPLABOR, Presidente Prudente, SP, Brazil) to a particle size of 10 mesh (2.0 mm), stored into plastic bags, and kept in a freezer (−8 °C), prior to pretreatments.

SCS samples were pretreated by hydrothermolysis in 5.5 L benchtop pressure reactor (model 4584, Parr Instrument Company, Moline, IL, USA) equipped with propeller agitator, heater, and temperature controller). Raw SCS samples were mixed with distillate water in a solid/liquid ratio of 1:10 (w/v) inside the reactor. The reactions were performed under 195 °C for 10 min. Further details concerning the pretreatment step can be found in Santos-Rocha et al. (2017). The biomass was characterized in terms of its chemical composition, according to the analytical procedures described by Sluiter et al. (2008), modified by Rocha et al. (1997), and validated by Gouveia et al. (2009). The enzymatic complex used in the assays was Cellic® CTec2, donated by Novozymes Latin America (Araucária, Paraná, Brazil). The enzymatic activity of this complex was 241 FPU/mL (Ghose, 1987) and 88.82 mg protein/mL (Bradford, 1976).

2.2. Batch and fed-batch strategies

The solid loadings and feeding strategies employed in the batch and fed-batch enzymatic hydrolyses are detailed in Table 1. All the hydrolysis experiments were made in duplicate.

Enzymatic hydrolysis experiments were conducted at solid loadings of 10, 20, and 30% (w/v), in batch and fed-batch modes. The enzyme loading adopted in this work (10 FPU/$g_{substrate}$, respectively) were defined in order to reach values of cellulose-to-glucose conversions above 60% in 72 h of enzymatic hydrolysis. The enzyme dosage was based on the values used by Angarita et al. (2015) and Corrêa et al. (2016a, 2016b). These assays were performed at 50 °C in stirred tank reactors with reaction volumes of 50 mL and 3 L, using 50 mM sodium citrate buffer (pH 4.8). The 50 mL reactor was operated for 72 h, with stirring at 250 rpm using three two-flat-blade paddle impellers. According to Pratto et al. (2016), agitation speeds up to 200 rpm minimize the effects of mass transfer in the hydrolysis reaction. The 3 L reactor was operated for 168 h, with stirring at 470 rpm using two three-blade elephant ear impellers. Three impellers were used for the hydrolysis assays in the 50 mL reactor in order minimize problems related to mass and heat transfer. The agitation speed and impeller configuration used for the hydrolysis experiments in the 3 L reactor was selected based on an optimized condition described in previous work of our research group

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