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Fed-batch strategies for saccharification of pilot-scale mild-acid and alkali pretreated sugarcane bagasse: Effects of solid loading and surfactant addition

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ABSTRACT

High-solid enzymatic hydrolysis of lignocellulosic biomass is a key approach for high titer and reduced downstream costs in second generation ethanol production. Combining a pilot-scale (80 L reactor) mild acid pretreatment and an alkali pretreatment of sugarcane bagasse (SCB) with fed-batch strategies for high-solid enzymatic hydrolysis, more than 150 g/L of glucose was found in the hydrolysate. The best result of the fed-batch study (2.03 g/L/h of glucose productivity) was obtained with a total solids content of 27%w/w (being 20%w/w of solids added at 0 h), and the addition of the total amount of the non-ionic surfactant PEG 4000 and the enzymes solely at the beginning of the reaction. A semi-simultaneous saccharification and fermentation strategy of the pretreated SCB (with the yeast inoculum occurring after 12 h of a single hydrolysis stage at most proper conditions for the cellulases) resulted in 62 g/L of ethanol after 48 h, and a productivity of up to 6.6 g/L/h. The strategy here reported indicates that it is possible to achieve ethanol titers suitable for industrial distilleries avoiding mass transfer limitations that incur in operational problems during high-solid enzymatic hydrolysis.

1. Introduction

Second generation bioethanol is a promising renewable fuel, since lignocellulosic materials are the most abundant biomass available, do not compete with edible crops, are usually less expensive than conventional agricultural feedstocks and can reduce greenhouse gas emissions (Kricka et al., 2015). In this context, the sugarcane bagasse (SCB) is one of the most abundant agroindustrial materials in the world, with an estimated generation in the last season (2016/2017) only in Brazil of circa 91 million tons (Unica, 2018; Castro and Castro, 2012).

Lignocellulosic feedstocks are mainly composed of cellulose, hemicellulose and lignin. The first two are sugar polymers that can be converted into fermentable sugars, mostly glucose and xylose (Sun and Cheng, 2002). Ethanol production processes include multiple steps: biomass pretreatment, enzymatic hydrolysis of the pretreated solid to release fermentable sugars, sugars fermentation to ethanol and distillation. Pretreatment is required to reduce cellulose crystallinity, and for partial removal of lignin and hemicellulose, turning biomass more accessible for enzymes during hydrolysis (Sarkar et al., 2012).

Enzymatic hydrolysis of the cellulose present in lignocellulosic materials depends on the synergistic activity of cellulases. Conversion yields depend on substrate and product concentration, ratio of distinct activities of cellulases in the complex, interaction of the enzyme with the biomass, mass transfer and, of course, enzyme dosage. High-solids hydrolysis and low enzyme dosage can be the keys for the economic feasibility of the process. However, this combination could lead to high viscosity, reduced mass transfer, and inhibition of enzymatic reaction due to unproductive adsorption, lignin negative interactions and product inhibition (Liu et al., 2016). The improvement of process configuration, by means for example of biomass fed-batch strategies, could overcome mass transfer problems, since new solid addition is made during liquefaction of the biomass initially added to the reaction, resulting in minimized content of insoluble solids in the medium (Sotaniemi et al., 2016). Another alternative for process improvement is the use of chemical additives, such as surfactants (e.g. PEG and Tween 80), which are known to diminish negative interactions of the enzymes with lignin (Liu et al., 2016).

High sugar concentration and high yield are crucial for economical production of lignocellulosic ethanol. Distillation cost is directly bound to ethanol concentration, and ethanol titers should be higher than 40 g/L to the process become viable (Zacchi and Axelsson, 1989). For cost reduction in downstream, a combination of high-solid pretreatment

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followed by high-solid (> 15%w/w) enzymatic hydrolysis process configurations has been considered (Sotaniemi et al., 2016; Wang et al., 2016a,b; Maeda et al., 2013; Gao et al., 2014). However, there are still opportunities to deepen investigation of the different possible process strategies, considering the single or the partitioned addition of the multiple components of the saccharification (substrate, enzymes, surfactant).

Therefore, in this study, we investigated fed-batch strategies of the different reaction components, aiming at to improve solids and sugar concentration in saccharification of mild acid and alkaline pretreated sugarcane bagasse.

2. Material and methods

2.1. Sugarcane bagasse pretreatment

The two-stages SCB pretreatment was carried out in a pilot batch reactor (80L working volume) provided with an internal screw (with an agitation speed of 40 rpm), located at the Petrobras pilot plants site (Rio de Janeiro, Brazil). In the first stage, SCB was soaked in a 1% (w/v) sulfuric acid solution for 30 min at 121 °C, under a solid:liquid ratio of 1:3. The acid-pretreated solid was pressed in a pilot hydraulic press (50 L nominal volume) at 90 bar. The pressed solid was then treated with a 3% NaOH (w/v) solution at 100 °C for 40 min in the same batch reactor of the first stage, pressed again, and finally washed until the liquid solution reached neutral pH (Sant'anna et al., 2005). The resulting material after the two pretreatment steps was named partially delignified cellulignin (PDC).

2.2. Enzymatic hydrolysis strategies

Enzymatic hydrolysis tests at high-solids content under either batch or fed-batch modes were carried out using Cellic CTec commercial cellulase preparation (kindly provided by Novozymes Latin America, Brazil). FPase activity (Filter paper activity) of this preparation was determined as 176 FPU/mL, according to the method described by Ghose (1986). Enzymatic hydrolysis was performed in duplicate in 500 mL shaken flasks, using 0.1 M sodium citrate buffer (pH 5.0) at 50 °C on a rotary shaker at 300 rpm for up to 144 h. The antibiotic Biozyn (Prozyn, Brazil) was used at a concentration of 50 ppm, to avoid contamination.

In a first sequence of tests (strategies I–IV), the aim was to evaluate the effect of partitioned addition of PDC or enzyme, and the effect of the addition of the non-ionic surfactant Polyethylene glycol 4000 (ULTRAPEG 4000 F, Oxiteno), named solely as PEG throughout the text (for conciseness). In these experiments, an initial PDC mass of 13.7 g was used, and when under fed-batch mode, portions of 3.4 g were added at each time indicated in Table 1. The enzyme loading was 26 FPU/g dry PDC at the beginning of each reaction. When enzyme fed-batch was investigated (strategy III), additional quantities corresponding to 26 FPU/g dry PDC added at the same time of enzyme addition were used.

Based on the results of the first tests, a second sequence of tests was designed (strategies V–VII), which focused primarily on the shortening of the intervals between the additions, as depicted in Table 1. The use of PEG was considered in all these cases, at a 5% (w/w dry PDC) content. The approaches resulted in different total solids (TS) content in each test, as shown along with the results. Additional explanation of the rationale behind the strategies is presented in the discussion of the results.

Table 1
Addition strategies used in the tests.

Strategy code	Addition strategy		
	SCB	Enzymes	PEG
I	t = 0 h	t = 0 h	–
II	t = 0, 8, 24, 48 h	t = 0 h	–
III	t = 0, 8, 24, 48 h	t = 0, 8, 24, 48 h	–
IV	t = 0, 8, 24, 48 h	t = 0 h	t = 0 h
V	t = 0, 4, 8 h	t = 0 h	t = 0, 4, 8 h
VI	t = 0, 4, 8 h	t = 0, 4, 8 h	t = 0, 4, 8 h
VII	t = 0, 4, 8, 24 h	t = 0, 4, 8, 24 h	t = 0, 4, 8, 24 h

After withdrawal, samples were centrifuged at 9000*g* at 20 °C for 5 min and the supernatants were filtered in a 0.20 µm porosity nylon membranes before analysis.

2.3. Semi-simultaneous saccharification and fermentation (sSSF)

The sSSF process was carried out in a jacketed and instrumented bioreactor with 1.3 L nominal volume (BioFlo 110, New Brunswick) provided with a pitched blade impeller. A previous enzymatic hydrolysis (namely pre-hydrolysis) phase during the first 12 h with fed batch of PDC and PEG (5% w/w) at 50 °C, pH 5.0 and 500 rpm was conducted. Then, the temperature and the agitation were reduced to 37 °C and 300 rpm (respectively) and *Sacharomyces cerevisiae* inoculum (commercial baker's yeast, brand Itaquara) was added at 10 g/L, aiming to study the fermentability of the resulting sugar solution, comprising a simultaneous saccharification and fermentation (SSF) phase. The total process time was 48 h, and no supplementation of the hydrolysate, with nutrients or carbohydrates, was done. The process was monitored in terms of release and consumption of carbohydrates and product generation (ethanol) by HPLC system, after samples centrifugation, as described in Section 2.2.

2.4. Analyses

SCB and PDC characterization was done in triplicate and was based on the NREL protocol TP-510-42618 (Sluiter et al., 2012). The total solids content of the wet PDC obtained after the pretreatments was determined in a thermogravimetric analyzer (MA-100, Sartorius).

For biomass observation by Scanning Electron Microscopy (SEM), samples were first washed twice with distilled water, dehydrated with ethanol and then freeze-dried. After that, they were placed on a metal plate and covered with a 30 nm gold layer using a JFC-1500 ion sputtering device (JEOL Co. Ltd., Japan) under argon atmosphere. Samples were then examined with a Quanta 200 scanning electron microscope (FEI, USA).

Glucose, cellobiose and ethanol concentrations during experiments were determined by HPLC system (Agilent 1260 Infinity), using a Bio-Rad Aminex HPX-87H column (kept at 65 °C) coupled with refractive index detector. The mobile phase was 0.005 mol/L H_2SO_4 at flow rate of 0.7 mL/min.

3. Results and discussion

3.1. Analysis of SCB and PDC

SEM micrographs (Fig. 1) show that after the sequential acid and alkali pretreatments the SCB fiber presented changes in structural morphology and fiber disorganization, resulting in lower alignment of the parallel stripes and the appearance of fractures. Similar morphological changes were observed by Rezende et al. (2011) after acid and alkali pretreatments of SCB. The morphological effects were, however, in a less extent to those found in SCB after pretreatment with the ionic liquid 1-ethyl-3-methylimidazolium acetate at 140 °C in a screw extruder (Silva et al., 2013).

Compositional analysis of the untreated SCB and the PDC showed 95% increase in glucan content with xylan and lignin reduction of 36% and 42%, respectively (Table 2). The pretreatments also substantially removed other components from hemicellulose fraction, as arabinan and acetic acid. The residual hemicellulose content in the pretreated