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Kinetics of enzyme-catalyzed hydrolysis of steam-exploded sugarcane bagasse

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HIGHLIGHTS

- The fractal kinetic model provided a good fit of the enzymatic hydrolysis data.
- Phosphoric acid is a better catalyst for steam explosion than sulphuric acid.
- Water washing is essential to remove inhibitors from steam-treated substrates.
- The fractal exponent revealed that alkali washing was not critical for hydrolysis.
- The fractal kinetics was useful to predict pretreatment performance.

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ABSTRACT

This work presents the experimental kinetic data and the fractal modeling of sugarcane bagasse steam treatment and enzymatic hydrolysis. Sugarcane bagasse (50 wt% moisture) was pretreated by autohydrolysis at 210 °C for 4 min. Acid catalysis involved the use of 9.5 mg g^{-1} of H_2SO_4 or H_3PO_4 in relation to the substrate dry mass at these same pretreatment conditions. Unwashed, water-washed and alkali-washed substrates were hydrolyzed at 2.0 wt% using 8 and 15 FPU g^{-1} (108.22 and 199.54 mg/g) total solids of a Celluclast 1.5 L and Novozym 188 mixture (Novozymes). The fractal kinetic modeling was used to describe the effect of pretreatment and both washing processes on substrate accessibility. Water and/or alkali washing was not strictly necessary to achieve high hydrolysis efficiencies. Also, the fractal model coefficients revealed that H_3PO_4 was a better pretreatment catalyst under the experimental conditions used in this study, resulting in the most susceptible substrates for enzymatic hydrolysis.

1. Introduction

In the past decade, important issues about the world climate change, along with the rising demand for renewable energy and the increased dependence on imported oil, have led several companies and research groups to develop alternative technologies for the production and use of liquid biofuels such as ethanol and biodiesel. In this scenario, short rotation herbaceous crops have been identified as one of the best sources for cellulosic ethanol mainly due to their high growth yield, suitable chemical composition and renewable characteristics (Soccol et al., 2010). One of such materials is sugar cane bagasse, an agro-industrial residue that is produced in large scale as a result of sugar and ethanol production in tropical countries like Brazil.

Lignocellulosic materials are mostly composed of cell wall polysaccharides (cellulose and hemicelluloses) from which fermentable sugars can be produced by acid or enzymatic hydrolysis and these can be converted to ethanol using suitable microbial strains. However, the plant cell wall was designed by nature to withstand biodegradation and, for this reason, the viability of cellulosic ethanol still relies on pretreatment and its full integration with other process operations such as enzymatic hydrolysis, fermentation and ethanol recovery (Cardona and Sanchez, 2007). A good pretreatment method must provide a considerable increase in cellulose accessibility to enzymatic hydrolysis while ensuring high recovery yields of the main components of the plant cell wall and a low generation of inhibitory compounds that are detrimental to fermenting microorganisms (Mosier et al., 2005; Ramos, 2003).

Steam explosion involves the pretreatment of lignocellulosic materials with saturated steam at 160–240 °C for reaction times ranging from 2 to 30 min in the absence (autohydrolysis) or

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presence of an acid catalyst (Brownell and Saddler, 1987; Brownell et al., 1986; Ramos, 2003). As a result of high pressure steaming, hemicelluloses and lignin are partially hydrolyzed to water-soluble monomers and oligomers while cellulose is partially modified in its crystallinity and degree of polymerization, therefore improving its susceptibility to solvation and enzymatic hydrolysis. However, this process can also generate biological inhibitors such as furan compounds and organic acids but most of these inhibitors can be eliminated from the fibrous material by simple water washing after pretreatment (Mosier et al., 2005; Ramos, 2003). As a result, fermentation of pretreatment water-solubles may require detoxification to bring the concentration of acetic acid, furfural and hydroxymethyfurfural down to non-inhibitory levels but this additional step increases the total energy consumption of the cellulosic ethanol production process considerably (Moncada et al., 2013). Also, removal of other inhibitory compounds such as phenolic acids may be carried out by extracting steam-treated materials with boiling solvents but this process also adds cost to the overall process (Li et al., 2013).

The cellulose present in pretreated lignocellulosic materials is difficult to hydrolyse due to both enzyme- and substrate-related factors. Substrate-related factors can be summarized as changes in its porosity and surface area, accumulation of lignin, and changes on cellulose crystallinity and degree of polymerization (Gupta and Lee, 2009; Nazhad et al., 1995). On the other hand, enzyme-related factors can be summarized as end-product inhibition due to glucose and cellobiose accumulation (Laser et al., 2002; Ramos and Saddler, 1994), thermal denaturation of enzymes after long periods of mechanical agitation (Azevedo et al., 2002), and irreversible adsorption of enzymes onto lignin and/or lignin-carbohydrate complexes (Emmel et al., 2003; Palonen et al., 2004).

The enzymatic hydrolysis of cellulose is usually carried out by a mixture of microbial (mostly fungal) hydrolases named "the cellulase complex", which is mainly composed of three different classes of enzymes: endo-β-1,4-glucanases (EG, EC 3.2.1.4), exo-β-1,4-glucanases or cellobiohydrolases (CBH, EC 3.2.1.91), and β-1,4-glucosidases (βG, EC 3.2.1.21). In fungal strains like *Trichoderma reesei*, these three classes of hydrolases act synergistically for the complete enzymatic hydrolysis of cellulosic substrates. First, cellulose reducing and non-reducing chain ends are formed by the action of EGs. Then, CBHs act at these chain ends releasing mostly cellobiose, with CBH I working progressively from the reducing ends and CBH II from the non-reducing ends. Finally, βGs complete this process by converting cellobiose to glucose (Reinikainen, 1994; Teeri, 1997).

Different kinetic models have been used to describe the enzymatic hydrolysis of cellulosic substrates (Gupta and Lee, 2009). Based on the fundamental approach and methodology used, these models can be divided in four classes: empirical models (Kim and Holtzapple, 2006), Michaelis–Menten models (Bezerra and Dias, 2004), adsorption models (Kadam et al., 2004), and models based on soluble substrates (Ting et al., 2009). Fractal kinetic models have also been used but not as thoroughly studied as other types of models (Wang and Feng, 2010). This method is particularly useful to study reactions that involve the diffusion of at least two catalytic species (for bimolecular reactions) on non-ideal substrate surfaces whose properties may lead to their partial segregation (Bansal et al., 2009). In general, the fractal exponent (h) is correlated to the structural organization of the substrate. Therefore, low *h* values are observed when the highest levels of plant cell wall deconstruction are achieved and this concept can be correlated with the extent of enzymatic hydrolysis of cellulosic substrates because this reaction involves a multicomponent water-soluble biocatalytic system acting on a rather heterogeneous substrate with variable porosity and available surface area (Xu and Ding, 2007).

In this work, a fractal kinetic model was employed to investigate the enzymatic hydrolysis of three different pretreated materials that were produced from sugarcane bagasse by auto-hydrolysis and acid-catalyzed steam explosion. The experimental data were treated to identify which pretreatment conditions were able to produce the highest hydrolysis efficiency among all substrates tested.

2. Methods

2.1. Materials

Sugarcane bagasse was obtained from a local sugarcane mill (Sucroalcooleira Melhoramentos, Paraná, Brazil), while the commercial enzymes used for hydrolysis were a gift from Novozymes (Bagsvaerd, Denmark). Celluclast® 1.5 L FG is a cellulase preparation produced by *T. reesei* whereas Novozym® 188 corresponds to a β-glucosidase preparation derived from *Aspergillus niger*.

All the reactants, solvents, chromatographic standards and laboratory supplies were obtained in analytical grade and used as received.

3. Experimental methods

Steam pretreatment was performed in a high pressure batch reactor at 210 °C for 4 min using sugarcane bagasse with a final moisture content of 50 wt%, without (autohydrolysis) and with previous impregnation with 9.5 mg acid/g dry bagasse of an acid catalyst (H_2SO_4 or H_3PO_4). Two washing processes were applied on the resulting pretreated material: water-washing to remove water-soluble hemicellulose and lignin components, and a water followed by alkali washing (sodium hydroxide 1 mol L^{-1} under reflux for 1 h at 5 wt%) to remove the alkali-soluble lignin as well as part of the existing lignin-carbohydrate complexes.

The total lignin content (acid-soluble plus acid-insoluble lignin) of substrates and raw materials was determined as recommended by Sluiter et al. (2008). The total carbohydrate content of cellulosic materials was determined by high performance liquid chromatography (HPLC) after acid hydrolysis to its component sugars. HPLC analysis was performed at 65 °C in Aminex HPX-87H column (Bio-Rad, Hercules, USA), eluted with 5 mmol L^{-1} H_2SO_4 at a flow rate of 0.6 mL min^{-1}. Quantification was performed by external calibration of the component sugars (cellobiose, glucose, xylose and arabinose), acetic acid and dehydration by-products (furfural and hydroxymethylfurfural).

All three different substrate types (unwashed, water-washed and alkali-washed) were hydrolysed at standard conditions (2 wt% total solids for 96 h at 45 °C and 150 rpm) using a 1:0.3 (vol/vol) mixture of Celluclast® 1.5 L FG and Novozym® 188 (Novozymes) containing different total cellulase activities, 8 and 15 FPU g^{-1} substrate (108.22 and 199.54 mg/g total solids), which were measured as filter paper units (FPU) (Ghose, 1987). Aliquots of approximately 1 mL were collected at 4, 8, 24, 48, 72 and 96 h, heated for 5 min in a boiling water bath, centrifuged at 10,000*g* and analyzed by HPLC as described above. In this case, the components monitored by HPLC were cellobiose, glucose and xylose. These HPLC values were subsequently converted to anhydrosugars and hydrolysis yields were calculated in relation to the amount of glucans (mostly cellulose) and hemicelluloses (mostly xylans) present in the original steam-treated substrate.

The total cellulase activity was determined against Whatman #1 filter paper according to Ghose (1987). All tests were carried out at 50 °C for 60 min in 50 mmol L^{-1} sodium acetate buffer, pH 4.8. One unit of filter paper activity (FPU) was defined as the amount of enzyme that was required to the release of