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Insight into the high-pressure CO_2 pre-treatment of sugarcane bagasse for a delivery of upgradable sugars

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This work provides an insight into sugarcane bagasse pre-treatment carried out with greener and more sustainable CO_2/H_2O system. Temperatures and residence times at a fixed initial CO_2 pressure were studied to verify their effects on pre-treatment efficiency with regard to the chemical composition of both water-soluble and water-insoluble fractions as well as to the susceptibility of the latter to enzymatic hydrolysis at high total solids. Also, trends in enzymatic hydrolysis were analysed in function of biomass crystallinity. This work provides an integrated approach in the analysis of upgradable sugars that are released as a result of pre-treatment and enzymatic hydrolysis. At optimal pre-treatment conditions, $17.2\,g\cdot L^{-1}$ sugars were released in the water-soluble fraction mainly as pentoses in monomeric and oligomeric forms. The enzymatic hydrolysis of solids produced at these pre-treatment conditions gave $76.8\,g\cdot L^{-1}$ glucose in the substrate hydrolysate. The overall sugar yield delivered in both pre-treatment and enzymatic hydrolysis was 73.9 mol%. These results were compared to the chemical effect of hydrothermal and/or physico-chemical effects of N_2-aided hydrothermal processes and showed that the greener processing of biomass pre-treatment with CO_2 is advantageous for the integrated valorisation of industrial residues and delivery of upgradable sugars within the biorefinery concept.

1. Introduction

Lignocellulosic biomass, such as forestry materials, grasses and agricultural residues (*e.g.* sugarcane bagasse), is one of the most promising renewable resources for the production of materials, chemicals and biofuels [1]. Aiming to convert lignocellulosic biomass into any of these products, a complex matrix of biopolymers including cellulose, hemicellulose and lignin must be disrupted into a stream of hexoses and pentoses, and valuable lignin derivatives. However, its successful implementation is hinged on the development of an efficient pre-treatment technique aiming at the production of sugars at affordable costs. To accomplish this aim, one of the key bottlenecks, which must addressed, is the recalcitrance of biomass. Recalcitrance refers to a structural resistance of plant cell walls to chemicals and enzymes that is conferred by ultrastructural, molecular and chemical properties [2]. Up to date, several pre-treatment techniques have been described to overcome this lignin-hemicellulose barrier prior to enzymatic action, and in some cases, to decrease the crystallinity of cellulose and its respective degree of polymerisation [3]. Unfortunately though, most of these pre-treatments are characterised by moderate selectivities and modest sugar yields, sometimes requiring overwhelming energy and/or chemical inputs [4]. These drawbacks, which often impose the economic viability of the entire process, have driven to the extensive development of new and advanced pre-treatment technologies [5]. One of them is the use of high-pressure CO_2/H_2O, in which the carbonic acid formed *in-situ* dissociates and leads to a progressive acidification of the reaction mixture. Consequently, the hydrolysis of hemicelluloses speeds up, especially over those found in hydrothermal pre-treatments where the initiator (carbonic acid) is absent, while leaving behind a cellulose-rich substrate that is more susceptible to enzymatic hydrolysis [6]. Furthermore, high-pressure CO_2/H_2O offers a possibility to produce a liquid stream without degradation products in

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such amounts as obtained in dilute-acid hydrolysis, since most of the volatile compounds that are inhibitory to fermentation can be removed during the depressurisation step. Previous works demonstrated that high-pressure CO_2/H_2O is an interesting alternative to dilute acid pre-treatment [7], and is an advance for hydrothermal processes that are already explored in industrial facilities. However, just a few strides have been made recently in elucidating what is the full advantage in using high-pressure CO_2/H_2O within biomass pre-treatment. Using a relatively small (160 mL) stirred reactor, the influence of temperature (180–220 °C) at high-pressure CO_2/H_2O on the enzymatic hydrolysis of sugarcane bagasse and elephant grass (*Pennisetum purpureum Schum.*) has been investigated. Glucose yields of 85.8% for sugarcane bagasse and 80.4% for elephant grass were obtained under similar operational conditions (220 °C and no residence time at the isothermal stage), with no extra catalysts and other chemicals added [8].

Despite the efficiency of high-pressure CO_2/H_2O in reducing the recalcitrance of lignocellulosic materials, it often requires high operational temperatures and pressures to speed up hemicellulose depolymerisation into oligomers, which can turn into by-products such as furfural. As result, the use of high temperatures creates significant economic and process challenges for the production of low-cost sugars. To improve the formation of total sugars under low temperatures, longer residence times have been applied in this study to improve CO_2 solubility and to avoid sugar degradation into unwanted by-products. Furthermore, the importance of integrated analysis of pre-treatment and enzymatic hydrolysis, as inseparable steps in the valorisation of sugarcane bagasse, was demonstrated. The pre-treatment effect was verified based on the formation of water-soluble sugars, on changes in crystallinity of the cellulose-rich leftover solids and on their susceptibility to enzymatic hydrolysis. In this context, upgradable sugars formed during pre-treatment and enzymatic hydrolysis were analysed together to express the overall sugar yields. Thus, concentrated solutions of easily upgradable sugars were achieved, which definitively contributes to the economics of this biomass conversion process.

2. Experimental section

2.1. Raw material preparation and chemicals

Sugarcane bagasse was obtained from the Cane Technology Centre – CTC (Piracicaba, Brazil). The biomass was reduced to particle sizes between 0.7 and 1.5 mm using a knife mill (IKA WERKE, Germany) in order to improve its homogeneity. CO_2 with a purity >99.99% ($w \cdot w^{-1}$) for the high-pressure CO_2/H_2O experiments was acquired from Air Liquide (Paris, France). Distilled water ($0.6\ M\Omega\, cm^{-1}$), used for the same purpose, was produced by the PURELAB Option Elga system. A 96% ($w \cdot w^{-1}$) H_2SO_4 delivered by Panreac Química (Barcelona, Spain) was used to prepare an aqueous solution of 72% ($w \cdot w^{-1}$) H_2SO_4 employed for chemical analysis of either untreated or pre-treated sugarcane bagasse. Paper filters (Ø = 150 mm, No. 1235) from Filter-Lab (Microchip Technology Inc., Arizona, USA) were used for post-processing filtrations. The enzymes used for hydrolysis (Cellic CTec3, batch VDNI1002 and Cellic HTec3, batch VIN10001) were kindly provided by Novozymes Latin America (Araucária, Brazil).

2.2. High-pressure CO_2/H_2O pre-treatment

The high-pressure CO_2/H_2O experiments were performed in a 250 mL stainless steel high-pressure batch reactor (series 4576B, Parr Instruments, Moline, USA) coupled to Parr 4848 control unit used to monitor and control temperature, pressure and stirring speed. The reactor was filled in with 14 g (dry basis) of sugarcane bagasse along with 140 mL of water to reach a fixed liquid-to-solid mass ratio of 10. CO_2 was loaded into the reactor at the initial pressure of 50 bar. The loaded gas had a temperature of −10 °C generated by passing CO_2 from the CO_2 high-pressure cylinder to the reactor through a 5 m ¼" i.d. loop immersed in a cooling bath containing a 1:1 mass mixture of monoethylene glycol and water. Right after the CO_2 loading, the reactor was heated then maintained at the desired temperature with a stirring speed of 200 rpm during time established by the design of experiments. After reaching the desired residence time, the reaction was quenched by rapid cooling of the reactor content to 25 °C. For this purpose, either an internal stainless-steel loop with continuously running tap water or a cold bath surrounding the reactor vessel was used. The reaction profiles (temperature and pressure as a function of time) are presented in Table 1S in Electronic Supplementary Information (ESI). The exhaust valve connected to the reactor was partially opened to slowly release CO_2 out of the system. This procedure allowed minimising losses of volatile by-products and helped to achieve a suitable mass balance for each fraction of the biomass used in the reactions. The pre-treated materials and the water-soluble fractions were separated by manual pressing. The recovered pre-treated materials were washed with 280 mL of distilled water and stored at 4 °C.

Using the optimal conditions (190 °C for 0 min and 160 °C for 15 min) that were established through to statistical design, which is presented in section below, control experiments with 50 bar of initial N_2 pressure and liquid hot water (water-only reaction in absence of CO_2 or N_2) were performed as described above. By definition, pre-treatment for 0 min translates into no residence time at the isothermal stage, or the time required to reach the desired temperature, when pre-treatment was stop by quenching the reactor content to 25 °C.

The mass balances were performed for the main biomass constituents and included the composition of both water-soluble and water-insoluble fractions. The composition of pre-treated solids was calculated as described elsewhere [9]. The yield of each product found in the water-soluble fraction ($Product\ yield\ (wt.\%)$) was calculated according to the following formula: $Product\ yield\ (wt.\%) = \frac{M_{product}/M_{fraction} \times [product]_{WSF} \times V_{reaction}}{m^{initial}_{biomass} \times fraction\ content^{\ initial}_{\ biomass}} \times 100$, where $M_{product}$ is the molar mass of the product, $M_{fraction}$ is the molar mass of the fraction originating the product, $[product\,]_{WSF}$ is the concentration of product in the water-soluble fraction in $g \cdot L^{-1}$ (determined according to method described in section 2.5), $V_{reaction}$ is the liquid phase reaction volume in L, $m^{initial}_{biomass}$ is the dry mass of sugarcane bagasse used in the reaction in g and $fraction\ content^{initial}_{biomass}$ in the pentosan, glucan or acetyl group content in relation to the dry mass of untreated biomass. Xylose, arabinose, furfural and formic acid were collectively considered in relation to the molar mass of sugarcane bagasse pentosan. For glucose and 5-hydroxymethylfurfural (5-HMF), the molar mass of glucan fraction was taken as the corresponding fraction, whereas for acetic acid, the molar mass of acetyl groups was considered in the above formula.

2.2.1. Design of experiments for high-pressure CO_2/H_2O and statistical analysis

A Doehlert statistical design of experiments (DoE) [10] was applied to assess the effect of temperature and residence time on overall sugar yields. Temperatures (X_1) between 160 and 200 °C and residence times (X_2) between 0 and 15 min were the independent variables scrutinised in this work and these were selected on the basis of previous literature reports [5]. The residence time means