Steam explosion pretreatment of oil palm empty fruit bunches (EFB) using autocatalytic hydrolysis: A biorefinery approach

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Highlights
- Analysis of pretreatment step using steam explosion under autocatalytic conditions.
- Erosion in OPEFB structures after steam explosion, improving the porosity and reducing the crystallinity of cellulose.
- Solubilisation of hemicellulose in form of xylose and arabinose.
- Enrichment of 24% in cellulose per gram of OPEFB treated.
- Production of liquid hydrolysate rich fermentable sugars.

Abstract
The oil palm empty fruit bunches (EFB) are an attractive source of carbon for the production of biochemical products, therefore, the aim of this work is to analyze the effect of the steam explosion (SE) pretreatment under autocatalytic conditions on EFB using a full experimental design. Temperature and reaction time were the operational variables studied. The EFB treated at 195°C for 6 min showed an increase of 34.69% in glycan (mostly cellulose), and a reduction of 68.12% in hemicelluloses, with increased enzymatic digestibility to 33% producing 4.2 g L⁻¹ of glucose. Scanning electron micrographs of the steam treated EFB exhibited surface erosion and an increased fiber porosity. Fourier transform infrared spectroscopy showed the solubilization of hemicellulose and modification of cellulose in treated EFB.

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1. Introduction
The production of oil palm from Elaeis guineensis in Brazil has increased in last decade, being the 10th producer in the world. However, 80% of oil palm in Brazil is used in the food industry. With the launching in January 2010 of the Brazilian National Program for the Production and Use of Biodiesel (PNPB), the inclusion of oil palm as feedstock for biodiesel production 31.8 million hectares (ha) of deforested area were identified in the Northeast, with good soil and climate conditions for palm oil cultivation. Moreover, the local installation and operation of almost 13 pilot plants improved the production capacity to 751 million of liters of oil per year.

Together with oil palm production, the generation of solid waste called oil palm empty fruit bunches, commonly referred as (EFB) is increasing. It is estimated that each 1 ton of oil palm produced generates 1.1 tons of EFB (Shinoj et al., 2011). This lignocellulosic biomass is mainly composed of cellulose (glucan), hemicellulose (xylan and arabinoxylans), and lignin. Being an attractive source for biofuels and value added chemicals it can be included in the energy matrix to improve the sustainability of palm oil biorefineries (Gnansounou and Dauriat, 2010; Noomtim and Cheirsilp, 2011).

Because of the recalcitrant nature of the lignocellulosic material found in EFB, a pretreatment stage is required to solubilize the hemicellulose and lignin and enhance the mass proportion of cellulose in the biomass. Pretreated material will have an increased porosity and improved accessibility toward the subsequent hydrolysis process, for the production of sugars useful for ethanol fermentation (Saikkku et al., 2012).

Steam explosion (SE) has been proved to be effective for a variety of lignocellulosic biomass, including hardwoods, softwoods, herbaceous residues, sugarcane bagasse, and wheat straw, being the most widely employed physico-chemical pretreatment for...
any lignocellulosic biomass (Fernandes et al., 2015; Ramos et al., 1992). During SE, the biomass is heated for a short time period with saturated steam at high pressure, followed by a sudden decompression. The high pressured steam provides an adequate temperature and reaction time as two main operational-variables. Changes in the mass composition of cellulose, hemicelluloses and acid insoluble lignin in solid fraction after SE were characterized and the liquid fraction was analyzed for the concentration of fermentable monomeric sugars and the generation of organic acids and/or inhibitory compounds. The effect of SE on EFB fiber was measured by the digestibility percentage, while the chemical modification of EFB was analyzed by Fourier Transform Infrared spectroscopy (FTIR) and the structure modification of EFB fibers was studied by scanning electron microscopy (SEM).

2. Methods

2.1. Raw material

Oil palm empty fruit bunches (OPEFB) were obtained from Bio-palma Vale factory, located in Moijú, Pará, state of Brazil. The OPEFB was dried in a cross flow stove at 65 °C for 72 h and stored in polyurethane bags at room temperature to avoid biological degradation.

2.2. Characterization of the EFB pretreatment fractions

The composition of the raw EFB and the solid fractions obtained after SE pretreatment, was determined according to the NREL analytical procedures reported by Sluiter et al. (2011). The cellulose (Cel) and hemicellulose (Hem) mass composition were calculated using the equations reported by Tan et al. (2013), in which the concentration of glucose (Gl), xylose (Xyl) and arabinose (Ara) are correlated. Analysis of acid hydrolysis was carried out by High-Performance Liquid Chromatography (HPLC), in a Shimadzu Chromatograph equipped with an Aminex HPX-87H column, working at 60 °C with sulfuric acid (5 mmol L⁻¹) as mobile phase at flow rate of 6 mL min⁻¹.

Furfural (F), hydroxymethyl furfural (HMF), acetic, formic and levulinic acid were determined in the pretreatment hydrolysates using a Shimadzu Chromatograph equipped with an Aminex HPX-87H and C18 columns at 60 °C. Mobile phase used was sulfuric acid (5 mmol L⁻¹) at rate of 6 mL min⁻¹ with an IR detector. Detection was carried out by differential refractometry and the quantification was based on external calibration as described by Scholl et al. (2015a).

The acid soluble lignin (ASL) and acid insoluble lignin (AIL) were determined as suggested in the NREL procedure (Sluiter et al., 2011). The acid soluble lignin was measured by UV spectroscopy at 280 nm using a SP – 2000 UV spectrophotometer with dilution factor of 10, while the acid insoluble lignin corresponded to the ash free residue that was obtained after sulfuric acid hydrolysis of plant polysaccharides.

2.3. Ash determination

The ash determination was carried out following the procedure reported by Sluiter et al. (2008) with little modifications. Briefly, 0.3 g of dry EFB were placed into a crucible and the samples were calcined at 555 °C for 6 h.

2.4. Protein determination

The quantitative determination of soluble proteins was performed using the protocol reported by Hames et al. (2008).

2.5. Extractives in ethanol and water

The determination of extractives components was carried out according to the NREL procedure reported by Sluiter et al. (2005). All experiments were done in triplicate.

2.6. Steam explosion pretreatment (SE)

The SE pretreatment was carried out in a stainless steel reactor with a 10 L capacity. The process flow diagram is presented at Fig. 1. Pretreatment was performed with 300 g of almost dried EFB, containing 2.0% ± 0.7% moisture. It has been demonstrated that humidity has no influence on the recovery of solid and liquid fraction obtained after SE (Pitarelo et al., 2012).

The material was introduced in the reactor vessel and saturated steam was fed until the desired temperature was reached, the heating time in all cases was around 2 min. The reaction time was controlled after the temperature was reached. The sudden decompression released the material into a cyclone and the vapor was liberated to the atmosphere. The pretreated material was washed five times with water (1 L) and solids were recovered by centrifugation. The first two washings were collected for analysis. The solids were oven dried and a fraction was milled for carbohydrate and lignin analyses.

2.7. Screening of pretreatment conditions

The influence of temperature and time on EFB pretreatment was evaluated initially at 160 °C and 212 °C for a fixed reaction time of 8 min. The EFB fibers derived from pretreatment were characterized in terms of cellulose, hemicellulose and lignin. The morphological changes were followed by SEM, with the aim to check the influence of the severity of different pretreatments on EFB.

2.8. Experimental design and statistical analysis

From the screening study, a full factorial design was implemented, with three level and two operational variables (³) temperature and reaction time, with three replicates at the center point to evaluate the system error. The recovery of solids and their mass composition in terms of cellulose, hemicellulose and acid insoluble lignin were expressed as response variables. The resulting values and statistical analysis were processed using Statistica Version 7.0 (Minneapolis, USA). Analysis of variance (ANOVA) was employed to determine statistical significance of the model. The experimental response obtained was analyzed with a second-order polynomial as is presented in Eq. (1). The summary of experimental design is presented in Table 2.

\[
y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \sum_{j=1 \neq i}^{n} \beta_{ij} X_i X_j
\]

where \(y\) is the response (cellulose, hemicellulose and acid insoluble lignin in fiber,%); \(X_i\) and \(X_j\) are the independent variables and \(\beta_0, \beta_i, \beta_{ij}\) are the intercept, linear, quadratic and interaction coefficients, respectively.
