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Combination of water extraction with dilute-sulphuric acid pretreatment for enhancing the enzymatic hydrolysis of *Jatropha curcas* shells

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ABSTRACT

Jatropha curcas shells were extracted with water in a pilot-scale reactor and then pretreated with dilute sulphuric acid. The pretreatment was initially investigated with a Box-Behnken experimental design in the range of 110–180 °C, 0.1–1.5% H_2SO_4 and 20–60 min, and then with complementary experiments at 190 °C. The glucan recovery was above 87% in all the experimental runs. Xylan solubilisation was 13–20% in the milder pretreatments and up to 45% in the most severe runs. Around 70% cellulose enzymatic conversion, evaluated with commercial cellulases during 72-h hydrolysis, was achieved for the pretreatments at 180 °C, and a region with maximal conversion was predicted for around 190 °C. For confirming that estimation, a 2^2-experiment augmented by one central point and parallel pretreatments of pre-extracted and non-extracted shells were performed. The highest cellulose conversion, reached at the central point, was 16.5% higher for the pre-extracted and pretreated material than for the directly pretreated one. The low cellulose crystallinity index (0.79) of the pre-extracted and pretreated shells correlated well with their better enzymatic convertibility.

1. Introduction

The decrease in the global reserves of fossil energy carriers has generated extensive research in recent years, and biodiesel has been identified as one of the options for at least complementing conventional fuels (Aransiola et al., 2014). Since the use of edible oils as raw materials could restrict the expansion of the biodiesel industry, alternative feedstocks, such as waste and non-edible oils, are considered for eliminating the competition with food consumption (Azam et al., 2005; Aransiola et al., 2014). The potential of different non-edible oilseeds has being investigated in Cuba during the last years within the BIOMAS-Cuba project (Martín et al., 2010; Suárez et al., 2011; Hernández et al., 2013; García et al., 2014), and *Jatropha curcas* is one of the most attractive options (Sotolongo et al., 2007). *J. curcas* is a tropical woody perennial oil-bearing tree species that may survive in harsh climate and soil conditions and its feasibility as a source of oil for biodiesel production has been demonstrated (Koh et al., 2011; van Eijck et al., 2014).

For facilitating the oil extraction, the seeds are separated from *J. curcas* fruits, and in that operation large amounts of residual shells, which represent almost one-third of the fruit's weight, are generated. Energy applications (Kratzeisen and Müller, 2009) and activated charcoal production (Ramakrishnan and Namasivayam, 2009) are potential uses for dealing with such a voluminous residue. *J. curcas* shells can also be considered as raw materials for producing ethanol (Visser et al., 2011). The *in situ* produced ethanol could be directed, together with the oil, to biodiesel production in an integrated configuration. As shown by Gutiérrez et al. (2009), the integration of ethanol and biodiesel production processes using a single source of biomass would allow a considerable reduction of the energy costs compared with the autonomous production of each of them.

For producing ethanol from lignocellulosic materials, such as *J. curcas* shells, their polysaccharides should be hydrolysed by means of acids or enzymes (Taherzadeh and Karimi, 2007). Enzymatic hydrolysis produces higher sugar yields and lower decomposition products than acid hydrolysis, but it requires a pretreatment step in order to make cellulose macromolecules accessible to enzymes

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(Yang and Wyman, 2008). Dilute-acid pretreatment has resulted effective for different materials, and it has high potential for industrial application. It is one of the methods favoured by the National Renewable Energy Laboratory (NREL), which is leading the largest cellulosic ethanol development effort in the world, because of its high recovery of hemicellulosic sugars upon pretreatment and its high enzymatic conversion of cellulose (Yang and Wyman, 2008).

The pretreatment and hydrolysis of *J. curcas* shells have been focus of recent investigations. Visser et al. (2012) reported the pretreatment at 121 °C with either 0.5% H_2SO_4 or 1% NaOH, while Marasabessy et al. (2012) reported the pretreatment of *J. curcas* shells in the range of 140–180 °C with 0.1–0.9% H_2SO_4 and showed that the highest glucan conversion can be achieved for the material pretreated at 178 °C, 0.9% H_2SO_4 and 30 min. In a recent study by us (García et al., 2014), it was found that the inclusion of an extraction step prior to the acid pretreatment improves the enzymatic conversion of cellulose. The current work aims to gain more insights into the dilute-acid pretreatment of water-extracted *J. curcas* shells.

2. Materials and methods

2.1. Materials

J. curcas fruits, harvested in May 2012 in a three-years old plantation (Indio Hatuey Experimental Station of Pastures and Forages, Matanzas, Cuba), were collected manually, air-dried to 85–90% dry matter content and peeled in a knife-mill (Retsch, SM 100, Haan, Germany). The shells were sun-dried for 3 days, milled to 2-mm particle size, sieved to remove the rejects, and kept in plastic bags at room temperature until further use. A small fraction of the shells was screened through a 1-mm sieve and used for the raw material analysis.

2.2. Water extraction of the raw material

Milled *J. curcas* shells corresponding to 2.3 kg dry matter (DM) were suspended in 24 L of water in a 100-L pilot-scale reactor (Herbst Machinenfabrik GmbH, Buxtehude, Germany). After filling and closing the reactor, it was moved to a horizontal position, and the suspension was heated to 100 °C and held for 1 h under slow agitation (5 rpm) with a system of paddles mounted in the central shaft. After that, the solid material was separated from the extract by filtration, and the filter cake was washed with abundant water, and dried for 5 days at room temperature until a final dry matter content of approximately 94%. A portion of the extractive-free material was submitted to compositional analysis, and the rest was used in the pretreatment experiment.

2.3. Pretreatment

Sixty grams (oven-dry basis) of extractive-free *J. curcas* shells were mixed with diluted H_2SO_4, at a liquid-to-solid ratio of 10:1, in 1-L stainless steel cylindrical reactors mounted on a rotary autoclave. A Box–Behnken response surface experimental design was applied to evaluate the effect of the temperature (110–180 °C), sulphuric acid concentration (0.1–1.5%) and pretreatment time (20–60 min) on the recovery of the main components and on the enzymatic hydrolysis (Table 1, rows 1–15). Additional pretreatments at 190 °C were also performed (Table 1, rows 16–24). The heating-up time ranged between 25 and 60 min depending on the pretreatment temperature. By the end of the pretreatment, the reactors with the pretreated material were cooled for around 20–30 min in a cold water bath. After that, the slurry was vacuum-filtered, and the pretreated solids were washed with abundant warm water. The pretreatment liquors were stored in plastic containers at 4 °C for analysis and further use, and the water washes were discarded. A portion of the pretreated solids was used for determination of the dry matter content (in triplicates), a second portion was directly placed in sealed plastic bags and stored frozen until enzymatic hydrolysis tests, and the rest was dried at room temperature in a climatisation chamber until a dry matter content of around 91%.

Table 1
Experimental conditions used for investigating the acid pretreatment of pre-extracted *J. curcas* shells.

Experimental run	Pre-extraction	Temperature (°C)	Time (min)	H_2SO_4 concentration (%)
1	Yes	110	20	0.8
2	Yes	110	40	0.1
3	Yes	110	40	1.5
4	Yes	110	60	0.8
5	Yes	145	20	0.1
6	Yes	145	20	1.5
7	Yes	145	60	0.1
8	Yes	145	60	1.5
9	Yes	145	40	0.8
10	Yes	145	40	0.8
11	Yes	145	40	0.8
12	Yes	180	20	0.8
13	Yes	180	40	0.1
14	Yes	180	40	1.5
15	Yes	180	60	0.8
16	Yes	190	20	0.1
17	Yes	190	20	1.5
18	Yes	190	40	0.8
19	Yes	190	60	0.1
20	Yes	190	60	1.5
21	No	190	40	0.8
22	No	190	40	0.8
23	Yes	190	40	0.8
24	Yes	190	40	0.8

Rows 1–15, Box–Behnken experimental design; rows 16–20, complementary experiment; rows 21–24, control experiment.

2.4. Enzymatic hydrolysis

One gram, on dry matter basis, of the washed pretreated material was suspended in 25 mL of citrate buffer (pH 5.0). A commercial preparation of *Trichoderma reesei* cellulases (Celluclast 1.5 L) and a β-glycosidase preparation (Novozym 188) (Novozymes, Denmark) were added up to loadings of 25 FPU g^{-1} and 0.46 CBU mL^{-1}, respectively. The reaction mixture was incubated in a hood (Certomat HK, Sartorius AG, Göttingen, Germany) at 45 °C and 150 rpm, for 72 h. At the end of hydrolysis, glucose was quantified by HPLC, and the enzymatic convertibility of cellulose was calculated. All experiments were performed in triplicate.

2.5. Analytical methods

The chemical composition of both raw and pretreated shells was determined by analytical acid hydrolysis of the extractive-free material, followed by quantification of the sugars by borate complex ion exchange chromatography (Puls, 1993). The sugars were separated on a MCI Gel CA08F (Mitsubishi) column at 60 °C, using a linear gradient of 0.3–0.9 M potassium borate buffer (pH 9.2) at a flow rate of 0.7 mL min^{-1} within 35 min. For spectrophotometric detection of sugars at 560 nm, derivatisation with cuprum bicinchoninate was performed. The content of cellulose and xylan was calculated based on the concentrations of glucose and xylose, respectively. Acid-insoluble lignin was determined gravimetrically, whereas the soluble lignin was determined spectrophotometrically at 205 nm, on an UV/VIS spectrophotometer (LAMBDA 650, Perkin Elmer LAS, Rodgau, Germany). Furfural and 5-hydroxy methylfurfural were analysed by reverse-phase high-performance