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Short Communication

Comparative study of various pretreatment reagents on rice husk and structural changes assessment of the optimized pretreated rice husk

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HIGHLIGHTS

- Among the pretreatment reagents, HCl hydrolyzed rice husk (RH) the best.
- The optimized pretreatment condition is mild compared with other similar studies.
- The increased pore volume & size of pretreated RH favors fungal fermentation.

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ABSTRACT

The performance of alkalis (NaOH and $Ca(OH)_2$) and acids (H_2SO_4, HCl, H_3PO_4, CH_3COOH, and HNO_3) in the pretreatment of rice husk was screened, and a suitable reagent was assessed for subsequent optimization using response surface methodology. From the assessment, HCl that hydrolysed rice husk well was optimized with three parameters (HCl loading, pretreatment duration, and temperature) using Box–Behnken Design. The optimized condition (0.5% (w/v) HCl loading, 125 °C, 1.5 h) is relatively mild, and resulted in ~22.3 mg TRS/ml hydrolysate. The reduced model developed has good predictability, where the predicted and experimental results differ by only 2%. The comprehensive structural characterization studies that involved FT-IR, XRD, SEM, and BET surface area determination showed that the pretreated rice husk consisted mainly of cellulose and lignin. Compared to untreated rice husk, pretreated rice husk possessed increased pore size and pore volume, which are expected to be beneficial for fungal growth during fermentation.

1. Introduction

Rice husk is a by-product of rice milling industry, and it represents approximately 20% by weight of rough rice (Hashim et al., 1996). In 2008, there were estimated 125 Mt and 137 Mt rice husk generated in Asia and worldwide, respectively (FAOSTAT, 2010). Rice husk contains relatively high cellulose content (40–60%), and it is widely available at relatively low cost. Attributing to its recalcitrant nature, direct conversion of untreated rice husk usually results in low product yield. Therefore, pretreatment is necessary to partially disrupt the recalcitrant structure to achieve delignification of the lignocellulosic biomass. This renders the solid substrate more accessible to enzyme or microorganism during bioconversion.

Prevailing rice husk pretreatments reported in literatures include acid (Dagnino et al., 2013), alkaline (Saha and Cotta, 2008; Singh et al., 2011), hydrothermal (Zheng et al., 2007), alkaline peroxide (Saha and Cotta, 2007), and ionic liquid dissolution pretreatments (Ang et al., 2011; Lynam et al., 2012). Among them, acid and alkaline pretreatments have been extensively used for pretreating lignocellulosic biomass (Kaar and Holtzapple, 2000; Saha and Cotta, 2008).

To date, no specifically effective reagent in pretreating rice husk has been reported. To have a greater insight into the pretreatment of rice husk, alkalis and acids were screened and assessed in the study, and the best performing reagent was subjected to subsequent optimization study. Various structural analyses including Fourier transform–infrared spectroscopy (FT-IR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and Brunauer–Emmett–Teller (BET) surface area determination were conducted to assess the compositional, chemical, and structural changes of the biomass that is imparted by the selected pretreatment.

2. Methodology

2.1. Materials

Rice husk was collected from Ng Trading Company, Selangor, Malaysia. The sample was washed and dried at 55 °C before being

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milled to approximately 30 mesh size, and was stored in a dry cabinet prior to use.

2.2. Assessment of pretreatment reagents

Screening was conducted on two alkalis and five acids, namely sodium hydroxide (NaOH, Merck), calcium hydroxide ($Ca(OH)_2$, Sigma–Aldrich), sulphuric acid (H_2SO_4, Fisher Scientific), hydrochloric acid (HCl, Merck), phosphoric acid (H_3PO_4, Ajax Chemicals), acetic acid (CH_3COOH, Riedel–de Haen) and nitric acid (HNO_3, Scharlau Chemie). With reference to the literatures (Chang et al., 1997; Saha and Cotta, 2008), the screening were carried out at the following conditions: (i) concentration of reagent, 0.5% (w/v); (ii) rice husk loading, 10.0% (w/v); (iii) water loading, 10.0 ml/g; (iv) pretreatment temperature, 100 ± 1 °C; (v) pretreatment duration, 2 h. Total reducing sugars (TRS) content was employed as an indirect pretreatment indicator, and its content in hydrolysate was determined by DNS method (Miller, 1959). The main action of acids and alkalis is to dissolve hemicellulose, to some extent cellulose, and lignin. Thus, TRS released from the hydrolysis of hemicellulose/cellulose can suitably reflect the extent of structure disruption in rice husk. Pretreatment reagent that releases the highest TRS in hydrolysate was selected for subsequent optimization studies. To investigate the compositional changes imparted by the pretreatment reagents, cellulose, hemicellulose, lignin, and ash contents of the pretreated rice husk samples were characterized by using the Association Official Analytical Chemists (AOAC, (2005)) official methods.

2.3. Optimization of pretreatment

The pretreatment reagent selected in Section 2.2 was employed in the optimization of rice husk pretreatment. Three parameters known to have effect on pretreatment, namely reagent loading (X_1), heating duration (X_2) and heating temperature (X_3), were investigated. To determine the low and high levels of the chosen parameters, preliminary tests were conducted at: (i) reagent loading, 0–4% (w/v); (ii) heating duration, 1–6 h; (iii) heating temperature, 60–140 °C. The tests were performed by one-factor-at-a-time approach, and TRS detected in hydrolysate was measured as response for optimization of pretreatment.

The range of each parameter determined from the preliminary tests was applied in the optimization study using Box–Behnken experimental design (BBD). TRS detected in the hydrolysate was determined as response (Y). The maximum release of TRS in hydrolysate was determined using response surface methodology (RSM), and the regression analysis of optimization data was performed with the aid of Design-Expert Version 6.0.6 (Stat-Ease Inc., Minneapolis).

2.4. Analytical techniques

The FT-IR spectra of the rice husk samples between 600 and 4000 cm^{-1} at 4 cm^{-1} nominal resolution were recorded at room temperature with a FT-IR/FT–FIR spectrometer (Perkin Elmer, Spectrum 400, USA). The spectra were presented in relative transmittance percentage (%) of wave number (cm^{-1}) and the background was recorded with empty cell.

The crystallinity of the rice husk samples was examined by XRD measurement performed with a D8 Advanced X-ray diffractometer (Bruker AXS, USA) using Cu Kα monochromatized radiation at 40 kV and 40 mA at ambient temperature. The samples were scanned and the intensities were recorded in 2θ range from 10° to 80° with a step size of 0.02°. The crystallinity index (CrI) of the rice husk samples was calculated by using equation as reported by Parikh et al. (2007).

The structural changes of the pretreated rice husk were assessed with scanning electron microscope Quanta™ 200 FESEM (FEI, USA) operated at 2–5 kV accelerating voltage under low vacuum.

The surface area and average pore size of rice husk samples were determined by nitrogen adsorption isotherm at 77 K using a high-performance six-sample surface area and pore size analyzer Autosorb®-6B (Quantachrome, Florida, USA). The nitrogen adsorption–desorption isotherm was operated at relative pressure P/P_0 of 0.3, where P is the system pressure and P_0 is the initial pressure at 1 bar.

3. Results and discussion

3.1. Assessment of pretreatment reagent

Based on the results of screening for suitable pretreatment reagent, each alkali and acid had pretreated rice husk to a varying extent. Low amount of TRS was detected in the hydrolysates of NaOH and $Ca(OH)_2$ pretreatments. This is because the alkalis only fractionally hydrolysed hemicellulose and cellulose (Weil et al., 1994), but mainly delignified rice husk (Brannvall, 2004). Generally, findings from the assessment show that acids were better pretreatment reagents than alkalis (Fig. A1). The highest TRS was detected in the hydrolysate of HCl pretreatment (15.0 ± 0.6 mg/ml), followed by HNO_3 (12.2 ± 0.1 mg/ml) and H_2SO_4 (7.2 ± 0.3 mg/ml). Pretreatment with CH_3COOH, H_3PO_4, NaOH, and $Ca(OH)_2$ produced less than 1 mg TRS/ml hydrolysate, which is similar to pretreatment using only water.

The characterization of rice husk samples showed that all acid-pretreated rice husk had reduced hemicellulose content (Fig. 1), which explains the acids main role were hydrolysing the amorphous hemicellulose in the substrate (Orozco et al., 2007). Besides, cellulose was also partially hydrolysed during the acid pretreatments of rice husk (Weil et al., 1994), particularly pretreatments with strong acids, such as HCl, HNO_3 and H_2SO_4. The availability of more reactive protons disrupt hydrogen bonding of cellulose chain prior to hydrolysis resulting in higher TRS yield (Orozco et al., 2007).

Among the reagents, HCl hydrolysed rice husk and released the highest amount of TRS during pretreatment. This signifies the effectiveness of HCl pretreatment in disrupting rice husk structure, while retaining significant amount of cellulose (~60%) in pretreated rice husk. Thus, HCl pretreatment was further optimized.

3.2. Optimization of pretreatment

The preliminary pretreatment study shows that TRS yield increased sharply in the first 2 h of pretreatment and reached plateau after 3 h (Fig. A2a). In HCl loading study, TRS yield increased sharply with HCl loading range between 0.25 and 0.75% (w/v) as more protons are available for the hydrolysis of rice husk (Fig. A2b). However, little increment in TRS yield was observed with HCl loading higher than 1.0% (w/v) signifies that the rate of hydrolysis is limited by the surface area of rice husk available for reaction. Furthermore, the TRS yield was found to increase proportionally with the pretreatment temperature (Fig. A2c).

The low and high levels of HCl loading (X_1), pretreatment duration (X_2), and temperature (X_3) were determined in the preliminary studies. The design matrix of BBD including the response (Y) is given in Table 1, where Y is the TRS detected in the hydrolysate. From the runs, the highest TRS (23.9 mg/ml) was obtained with pretreatment conditions at 0.75% (w/v) HCl loading, 120 °C for 2 h. The least TRS (10.9 mg/ml) was detected when pretreatment was conducted at 0.25% (w/v) HCl loading, 100 °C for 2 h. Only minute

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