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A novel combined pretreatment of ball milling and microwave irradiation for enhancing enzymatic hydrolysis of microcrystalline cellulose

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HIGHLIGHTS

- Bioconversion potential on MCC was evaluated with a new pretreatment method.
- An equation correlating glucose yield with CrI, SSA and DP was deduced.
- The rate of enzymatic hydrolysis was much more sensitive to CrI than SSA and DP.
- Combination of BM and short time MWI is a feasible approach to treat biomass.

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ABSTRACT

Microcrystalline cellulose (MCC) was performed as a mode substrate to investigate its potential ability of bioconversion in a novel combined pretreatment of ball milling (BM) and/or microwave irradiation (MWI). The variation of structure characteristics of MCC before/after pretreatment were investigated, including crystallinity index (CrI), size of crystal (S_C), specific surface area (SSA) and degree of polymerization (DP). Their correlation with the rate of enzymatic hydrolysis was differentiated by an optimized equation which indicated the rate of hydrolysis was much more sensitive to CrI than SSA and DP. To achieve the same or higher glucose yield of BM for 3 h and 6 h, BM for 1 h with MWI for 20 min could save 54.8% and 77.40% energy consumption, respectively. Moreover, chemicals were not required in this process. It is concluded that the combination of BM and short time MWI is an environment-friendly, economical and effective approach to treat biomass.

1. Introduction

Excessive consumption of fossil fuels has resulted in emission of high levels of pollutants such as greenhouse gases during the last few decades. Biomass such as rice straw, sugarcane bagasse and corn straw are attractive feedstock for the production of alternative fuels and chemicals, which are used to being refined from petroleum (Sarkar et al., 2012). But due to the complex structure of biomass, the unit operations of the biorefinery are expensive, low-effective and cause environment pollution, which limit the development of biomass energy. Pretreatment unit is one of the most expensive units (Laser et al., 2002), which arouse the concerns of researchers. At the same time, many pretreatment technologies have already been developed, such as steam explosion, ammonia fiber explosion (AFEX), liquid hot water treatment, biological treatment, ball milling, microwave irradiation (MWI), ionic liquid treatment, etc. However, these technologies suffer

Abbreviations: B, full width half maximum (FWHM) of the reflection measured in 2θ corresponding to Bragg angle; BM, BM1, BM3, BM6, ball milling, ball milling for 1 h, 3 h and 6 h, respectively; BMMWI, ball milling followed by microwave irradiation; BM1MWI, ball milling for 1 h followed by microwave irradiation; C_G, concentration of glucose, g/L; CrI, crystallinity index; DP, degree of polymerization; G_{MC}, glucosyl monomer concentration, μg/mL; G_Y, glucose yield of the theoretical, %; G_{6Y}, glucose yield of the theoretical after 6 h, %; I_{002}, maximum intensity above base line at $2\theta = 22°$; I_{Amorph}, minimum intensity above base line corresponding to amorphous content at $2\theta = 18°$; k, Scherrer constant (0.84); MWI, microwave irradiation; M_{PS}, mass of solid fraction after pretreatment, g; M_R, mass of raw feedstock, g; M_P, mass of pretreated MCC, g; R_{EC}, reducing-end concentration, μg/mL; R_S, solid recovery after pretreatment, %; S_C, average size of crystal; V_E, volume of the enzymatic reaction mixture, L; λ, X-ray wavelength, 1.54 Å; ϑ, diffraction angle, °.

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from relatively low sugar yields, severe reaction conditions and high processing costs (Chen et al., 2012). It is necessary to develop new approaches to treat biomass.

Ball milling (BM) was effective in reducing the crystallinity index (CrI) (Howsmon and Marchessault, 1959; Silva et al., 2012) which greatly improved the accessibility of the reactants and catalysts to the β-1,4-glycosidic bonds (Zhao et al., 2006). Besides, no effluent produced in the process but with the improvement of reactivity of biomass. Short time BM could change the CrI and/or DP of biomass (Schwanninger et al., 2004), improve the accessibility of aspen wood to cellulase (Fukazawa et al., 1982). The digest ability of corn stover was greatly increased by wet alkaline BM, which was much better than dry BM (Lin et al., 2010). However, BM treatment suffers from the high energy consumption, which hinders the scale-up of the BM technology into industry.

Compared with conduction/convection heating, which is based on superficial heat transfer, microwave irradiation (MWI) uses the ability of direct interaction between a heated object and an applied electromagnetic field to create heat. Thus, MWI is highly effective and energy saving (Gabhane et al., 2011). Microwave irradiation was successfully applied in biomass pretreatment because of either non-thermal or thermal effects, which caused fragmentation and swelling, leading to degradation of lignin and hemicellulose in the biomass (Chen et al., 2011a). In order to release more monosaccharide and reducing sugars, the addition of chemicals was necessary in MWI-assisted pretreatment of biomass, such as sugarcane bagasse, *Miscanthus sinensis*, sorghum, wheat straw. Nevertheless, the addition of chemicals will bring the pollution and increase the cost of the pretreatment technology.

Therefore, BM or MWI alone was not adequate to convert biomass to fermentable sugars with a high yield in an economical and environment-friendly way. The combination of BM and MWI (BMMWI) might provide a more economical practical pretreatment technology. In the present study, with the objective of developing a novel environment-friendly and high-effective approach to treat biomass, the digest ability of MCC treated by BM and/or MWI was investigated. Besides, characteristics of untreated and pretreated MCC including CrI, Sc, SSA, DP were determined and their relationship with the rate of enzymatic hydrolysis was analyzed.

2. Methods

2.1. Feedstock preparation

Two kinds of microcrystalline cellulose (MCC): VIVAPUR®105 and VIVAPUR®12, were purchased from JRS (Rosenberg, Germany). After being selected from the above two MCC by different size of sieves, the feedstocks with 180–200 μm, 25–40 μm were prepared and labeled as MCC01 and MCC04, respectively.

2.2. Ball milling treatment

A WL-IA planetary ball mill (Rishengjiuyuan Co. Ltd., Tianjing, China) and four zirconia milling cylinders (500 mL) were used. The mechanism of the WL-IA planetary BM is as following: triangle belt is adopted to transmit the BM, of which the disk that equipped with four cylinders is drove by an alternating current dynamo. Revolution begins when the disk moves, meanwhile, the four cylinders rotate around the axis of the disk in the reverse direction. In the process of revolution and rotation, the balls and samples collide with each other with centrifugal force, which make the samples grind to small pieces. The MCC of 30 g dry matter and 50 zirconia balls (φ = 10 mm) were placed into each cylinder, up to about one third of its capacity. The mill rotated horizontally at constant speed of 1000 rpm for different times. The samples after BM for 1 h, 3 h, 6 h were labeled as BM1, BM3, BM6, respectively, and then were sealed for the following experiments, which were performed in duplicate.

2.3. Microwave irradiation treatment

MCR-3 microwave chemical reactor (Yuhua Equipment Co. Ltd., GongYi, China) was employed in the process of the MWI treatment. The reactor is a batch type with a stainless steel vessel, equipped with a stirrer and a thermocouple. The maximum output power is 800 W and that can be adjusted automatically by the inner temperature of the reactor. The pretreatment was done with the liquid to solid ratio of 50:3 (w/w), 20 min, 800 W and 200 rpm. The mixture was placed in a beaker with a glass cover under MWI.

After MWI treatment, the mixture was almost evaporated to dryness, and the solid residue was collected. Some of the solid residue was weighed for calculating the solid recovery rate (R_S), the left was then stored at 4 °C for the enzymatic hydrolysis. All the experiments were performed in duplicate. The R_S was calculated as following:

$$R_S = \frac{M_{PS}}{M_R} \times 100\% \quad (1)$$

2.4. Enzymatic hydrolysis

A mixture of 1 g dry matter (DM) MCC, 0.1 M 50 mL citrate buffer (pH 4.8), 1 mL NaN_3 (2%, m/v), cellulase with 35 FPA/g DM and two small glass balls was used in the process of the enzymatic hydrolysis. The final weight was brought to 100 g with the addition of tap water and the pH was adjusted to 4.8. Cellulase with FPA of 30 FPA/mL was purchased from Zesheng Bioengineering Technology Co., Ltd., China. The enzymatic hydrolysis was performed at 50 °C and 200 rpm. An aliquot (1 mL) was withdrawn at different time intervals and filtered with 0.45 μm membrane for the analysis of sugars. The experiment plan is showed in Fig. 1. All reactions were performed in duplicate. Glucose yield of the theoretical (G_Y) was calculated as following:

$$G_Y\,(\%) = \frac{C_G \times \frac{9}{10} \times V_E}{M_P \,\text{or}\, M_R} \times 100\% \quad (2)$$

2.5. Analysis methods

2.5.1. Dry matter content

The moisture analyzer, Mettler Toledo HR83 was employed in the determination of dry matter content. Duplicate experiments were run for each sample.

2.5.2. Crystallinity measurement

An X'Pert PRO MPD X-ray diffractometer was used to record the X-ray diffraction pattern at 25 °C from 10° to 60°, using Cu/Kα1 irradiation (1.54 Å) at 40 kV and 40 mA. Scan speed was 0.02°/s with a step size of 0.0170° (Bansal et al., 2010). Each analysis was repeated twice.

The empirical peak-height method proposed by Segal (Segal et al., 1959) calculating CrI was applied:

$$\text{CrI} = \frac{(I_{002} - I_{\text{Amorph}})}{I_{002}} \times 100\% \quad (3)$$

The average size of crystallite (S_C) was calculated by the Scherrer equation (Gumuskaya and Usta, 2006). And it was based on the width of the diffraction patterns occurred in the X-ray reflected crystalline region.

$$S_C = \frac{k \times \lambda}{B \times \cos\theta} \quad (4)$$