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Depolymerization of microcrystalline cellulose by the combination of ultrasound and Fenton reagent

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ABSTRACT

In this study, the combined use of Fenton reagent and ultrasound to the pretreatment of microcrystalline cellulose (MCC) for subsequent enzyme hydrolysis was investigated. The morphological analysis showed that the aspect ratio of MCC was greatly reduced after pretreatment. The X-ray diffraction (XRD) and degree of polymerization (DP) analyses showed that Fenton reagent was more efficient in decreasing the crystallinity of MCC while ultrasound was more efficient in decreasing the DP of MCC. The combination of Fenton reaction and ultrasound, which produced the lowest crystallinity (84.8 ± 0.2%) and DP (124.7 ± 0.6) of MCC and the highest yield of reducing sugar (22.9 ± 0.3 g/100 g), provides a promising pretreatment process for MCC depolymerization.

1. Introduction

In recent years, conversion of biomass has attracted increasing attention mainly driven by growing concerns about the depletion of fossil fuels and environmental problems stemming from greenhouse gas emissions. Cellulosic biomass represents a huge reservoir of renewable carbon and the conversion of cellulose, and more largely lignocellulosic biomass, to biofuels or platform chemicals has received considerable attention [1–3]. In all these processes, the depolymerization of cellulose to glucose is a prerequisite step from which biofuels and platform chemicals are then produced [4].

The depolymerization of cellulose to glucose is a highly difficult task owing to its crystalline structure making it recalcitrant to hydrolysis. To make cellulose more susceptible to hydrolysis, physical, chemical, physiochemical and biological pretreatment technologies have been developed to reduce the effect of the limiting factors such as degree of polymerization (DP) and crystallinity of cellulose on its hydrolysis [5]. Of the various pretreatment technologies, biological pretreatment is probably the most economical one because it bears the advantages of low energy requirement and mild environmental conditions. In biological pretreatment processes, microorganisms such as white- and brown-rot fungi are used to depolymerize cellulose.

Halliwell in 1960s found that the cellulose degraded by OH radicals produced by Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {\cdot}OH + OH^-$) resembled that degraded by microorganism [6]. Later, some researches confirmed that cellobiose dehydrogenase produced by brown- and soft-rot fungi could decrease the DP and destroy the crystalline structure of cellulose by generating OH radicals through Fenton reaction [7–10]. It is believed that the highly reactive electrophilic OH radicals could react with cellulose by abstracting a hydrogen atom, resulting in formation of reactive organic radicals, leading to depolymerization and changes in fiber surface topography [11]. Although Fenton-based biological pretreatment has the advantages of low energy requirement and mild environmental conditions, it is very time-consuming [12], which may result from the low concentration of OH radicals generated during the pretreatment process. Therefore, utilizing Fenton-based technology to generate OH radicals with suitable concentration for cellulose depolymerization deserve further investigation.

Depolymerization of cellulose by Fenton reagent has received great attention in recent years. Jain et al. evaluated the effectiveness of Fenton's reagent as a pretreatment agent on cotton cellulosic substrates and found that Fenton's pretreatment influenced the hydrolysis response of cellulase enzyme in a positive way [11]. Kato et al. found that the solution phase Fenton chemistry was a viable pretreatment method to make cellulose more

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bioavailable for microbial bioethanol conversion [13]. Jung et al. employed Fenton's reagent for pretreatment of rice straw and obtained an enzymatic digestibility that was 93.2% of the theoretical glucose yield [1].

Ultrasound treatment of cellulose has emerged recently and received considerable attention [4]. Ultrasound produces its effects mainly through cavitation, which occurs at a very large number of sites in the reactor simultaneously and generates high temperature and pressure as well as intense shear forces, shock waves and microjets [14]. The decomposition of water molecules into extremely reactive radicals such as ·HO and ·H by high temperature and pressure resulting from cavitation aids in cleaving cellulose, resulting in decreased DP and crystallinity of cellulose and increased surface area [15]. The intense shear forces, shock waves and microjets resulting from cavitation can also facilitate cleavage of cellulose and enhance mass transfer through streaming within the solution [5].

Combined pretreatment processes for cellulose depolymerization have been recently considered as a promising approach to overcome drawbacks demonstrated by various individual pretreatment processes, by increasing efficiency of sugar production, decreasing formation of inhibitors and/or shortening process time [16]. Given that both Fenton reagent and ultrasound can depolymerize cellulose, combining Fenton reagent with ultrasound may provide a promising alternative pretreatment process for cellulose depolymerization. In this work, Fenton reagent, ultrasound, and the combination of Fenton reagent and ultrasound were evaluated as pretreatment to depolymerize microcrystalline cellulose (MCC) for subsequent enzyme hydrolysis, and the effect of the pretreatment was evaluated by measuring the cellulase enzyme activity response.

2. Methods

2.1. Materials

MCC of analytical grade was purchased from Sinopharm Chemical Reagent Co., Ltd. and grinded to pass through a 160-mesh sieve. Cellulase (⩾15,000 U/g) was purchased from Sinopharm Chemical Reagent Co., Ltd. Citric acid, $FeSO_4{\cdot}7H_2O$, H_2O_2, NaOH and HCl of analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd. All solutions were prepared in deionized water.

2.2. Pretreatment of MCC

In a typical Fenton pretreatment process, 6 g of MCC was added to a magnetically stirred flask (500 mL) containing 300 mL of H_2O kept at 25 °C, and 0.6 mL of H_2O_2 and 0.01 g of $FeSO_4{\cdot}7H_2O$ were added into the above suspension every 0.5 h for 5 times.

In a typical ultrasound pretreatment process, 6 g of MCC was added to a flask (500 mL) containing 300 mL of H_2O, and the suspension was subjected to sonication produced by a horn-type ultrasonic processor (VOSHIN-501D, Wuxi Voshin Instruments Co., Ltd.) with a power of 800 W, a frequency of 21–23 kHz, and an on-time of 2 s and an off-time of 4 s. The ultrasonic processor was equipped with temperature control and the temperature of the suspension was kept at 25 °C.

In a typical ultrasound/Fenton reagent pretreatment process, 6 g of MCC was added to a flask (500 mL) containing 300 mL of H_2O, and the suspension was subjected to sonication identical to that in the above ultrasound pretreatment process. During the pretreatment, 0.6 mL of H_2O_2 and 0.01 g of $FeSO_4{\cdot}7H_2O$ were added into the above suspension every 0.5 h for 5 times.

In each case, after 2.5 h of pretreatment, the resultant suspension was filtered. The obtained residual solid (pretreated MCC) was vacuum dried at 80 °C overnight. Raw MCC, Fenton pretreated MCC, ultrasound pretreated MCC, and ultrasound/Fenton reagent pretreated MCC were denoted as R-MCC, F-MCC, U-MCC, and U/F-MCC, respectively.

2.3. Enzyme hydrolysis

Briefly, 1.5 g of MCC (R-MCC or pretreated MCC) was first added to 30 ml of citric acid-NaOH buffer with a pH value of 4.8, then 0.07 g of cellulase was added to the above suspension, and the mixture was incubated in a shaker bath at 50 °C and 150 rpm for 48 h. The resultant mixture was filtered and the filtrate was collected for reducing sugar analysis.

2.4. Analytical methods

The morphologies of MCC before and after pretreatment were characterized by a polarization microscope (XP-330C, Shanghai Caikon Optical Instrument Co., Ltd.). Fourier transform infrared spectroscopy (FT-IR) analysis of MCC was performed on a Thermo Nicolet 6700 spectrometer over the wavenumber of 4000–400 cm^{-1}, with detector at 0.4 cm^{-1} resolution and 64 scans. The average DP of MCC was determined (25 °C) by copper ethylenediamine (CED) solution method, and triplicate runs were carried out. X-ray diffraction (XRD) patterns of MCC were recorded on an X-ray diffractometer (BRUKER D8 Advance) from 5° to 60° (2θ) using Cu Ka radiation (0.15418 nm) generated at 40 kV and 40 mA, and the scan speed was 0.01279°/s with a step size of 0.01944°. The crystallinity index was calculated from Eq. (1) according to the conventional peak intensity method,

$$I_{CR} = \frac{I_{002} - I_{am}}{I_{002}} \quad (1)$$

where I_{CR} denotes the crystallinity index, I_{002} the peak intensity at the plane (002) in the XRD profile, and I_{am} the intensity at 2θ value of 18°. The amount of reducing sugar released by enzyme hydrolysis was determined by the dinitrosalicylic acid (DNS) method with glucose as a standard [17]. The above XRD and DNS analyses were carried out in triplicate.

3. Results and discussion

3.1. Morphologies of MCC

Polarization microscope was employed to characterize the morphologies of MCC before and after pretreatment. It can be seen from Fig. 1(a) that R-MCC has a diameter of ca. 50 μm and an aspect ratio of ca. 30. It clearly shows that both Fenton reagent (Fig. 1(b)) and ultrasound (Fig. 1(c)) have a direct impact on the morphologies of MCC, as evidenced by the much reduced aspect ratio of the pretreated MCC. The decrease in aspect ratio of MCC after pretreatment, which suggests a disintegration of large MCC particles, is expected to favor a better contact with enzymes during the hydrolysis process and thus to enhance its reactivity [18]. The depolymerization of MCC by Fenton reagent could be attributed to the generated OH radicals, which can react with MCC by abstracting a hydrogen atom, resulting in depolymerization and changes in morphology of MCC [11]. The depolymerization of MCC by ultrasound, a process known as sonofragmentation [19], could be attributed to the cavitation effect. Ultrasound can generate alternating rarefaction and compression half-cycle. During the compression half cycle, certain sizes of cavities (bubbles) in the liquid medium are suddenly collapsed, creating powerful shock waves, and