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Short communication

Impact of regeneration process on the crystalline structure and enzymatic hydrolysis of cellulose obtained from ionic liquid

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ABSTRACT

The present study investigated the impact of regeneration process on the crystalline structure and enzymatic hydrolysis behaviors of microcrystalline cellulose (MCC) regenerated from ionic liquid 1-butyl-3-methylimidazolium chloride. The crystalline structures of these regenerated samples were analyzed by X-ray diffraction. Results suggested that almost amorphous cellulose was obtained by regenerating MCC in acetone (DRC-a), while partial cellulose II structure could be found in these regenerated samples from water and ethanol. Additionally, the enzymatic hydrolysis behaviors of MCC and its regenerated samples were comparatively studied. Results showed that above 90% of cellulose could be converted into glucose within 4 h for DRC-a and regenerated cellulose without drying (WRC-w) as compared to that of MCC (9.7%). Therefore, the regeneration process could significantly influence the crystallinity and digestibility of cellulose.

1. Introduction

Due to the contradiction between the increasing energy demands and limited fossil fuels, increasing attention has been paid to the sustainable conversion of biomass into valuable fuels or chemicals.

As the most abundant, renewable and non-edible natural polysaccharide in the world, cellulose is the most attractive resource for the production of bioethanol by enzymatic hydrolysis. However, long enzymatic hydrolysis time is always required to achieve a high conversion ratio. The enzymatic hydrolysis can be influenced by many factors, such as available surface area, crystallinity and polymerization of cellulose, content and distribution of lignin and hemicelluloses, etc (Alvira, Tomás-Pejó, Ballesteros, & Negro, 2010; Yeh, Huang, & Chen, 2010). However, previous work showed that without pretreatment the enzymatic hydrolysis rate of pure cellulose (e.g. cotton, MCC, bacterial cellulose) was relatively low even without the influence of lignin and hemicelluloses (Stauner, Silva, El Seoud, Frollini, & Petri, 2013; Xiao, Yin, Xia, & Ma, 2012). It indicated that the crystallinity and morphology of cellulose could significantly impact its enzymatic hydrolysis. Recently, ionic liquid was found to be an excellent solvent for cellulose (Zhu et al., 2006). The high polarity of ionic liquid could interrupt the compact hydrogen bond network of cellulose crystal at suitable temperature and resulted in a homogeneous cellulose solution, which was used to pretreat cellulose to change its crystallinity, morphology and available surface area (Cheng et al., 2011). However, crystalline cellulose II structure could always be found in their regenerated samples and thus partly affect the enzymatic hydrolysis rate of cellulose (Lozano, Bernal, Recio, & Belleville, 2012; Zhang, Wu, Zhang, & He, 2005). Therefore, to further improve the enzymatic hydrolysis rate of cellulose, avoiding the reforming of hydrogen bonds during the regeneration processes might be an alternative method.

In this work, regeneration process was mainly consisted of regeneration and drying process. Different solvents and drying methods were used to regenerate cellulose from ionic liquid. Since partial regenerated cellulose would recrystallize into cellulose II, the XRD patterns of all samples were investigate. Furthermore, the enzymatic hydrolysis behaviors of these samples were collected to evaluate the digestibility of these samples.

2. Materials and methods

2.1. Materials

Microcrystalline cellulose (MCC) was purchased from Sinopharm Chemical Reagent Company. 1-Butyl-3-methylimidazolium chloride (BMIMCl, purity > 99%) was obtained

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from Shanghai Chengjie Chemical Co., Ltd. Cellulase from *Trichoderma reesei* ATCC 26921 and Cellobiase from *Aspergillius niger* were purchased from Sigma–Aldrich. Cellulase activity (170 FPU mL^{-1}) and cellobiase activity (560 IU mL^{-1}) were determined by the standard IUPAC method (Ghose, 1987). Other chemicals were all analytical reagents.

2.2. Preparation of regenerated cellulose from BMIMCl

Firstly, a 4% (w/w) cellulose solution was prepared by combining 100 g of BMIMCl with 4.0 g of MCC into a flask equipped with a magnetic stirrer. The mixture was stirred at 110 °C for 2 h until MCC was fully dissolved. Then, 100 mL of dimethyl sulfoxide (DMSO) was added into the viscous mixture to reduce its viscosity. The cellulose was regenerated by adding the mixture into water, ethanol or acetone solution under vigorously stirring. The regenerated cellulose was recovered by filtration through a nylon membrane (0.1 mm mesh). Then, the regenerated samples were dialyzed in their corresponding solvents at room temperature for three days. The cellulose samples regenerated from water, ethanol and acetone were dried in a vacuum oven at 60 °C overnight and labeled as DRC-w, DRC-e, DRC-a, respectively. The cellulose regenerated from water and dried in a freeze drier was labeled as FDRC-w. Additionally, some gelatinous cellulose regenerated from water was washed thoroughly with 50 mM citrate buffer containing 0.03% (w/v) sodium azide (pH 4.8) and labeled as WRC-w.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was conducted as follows: reaction conditions were 50 °C, 10 mL of 50 mM sodium citrate buffer containing 0.03% (w/v) sodium azide (pH 4.8), 2 wt% substrate, with incubation in an air bath shaking incubator at 150 rpm. Cellulase (17 FPU/g substrate) and cellobiase (34 IU/g substrate) was mixed and used for all hydrolysis experiments. The enzyme hydrolyzates were collected as a function of time for further analysis.

2.4. Analysis methods

X-ray diffraction (XRD) analysis of MCC and regenerated cellulose were performed on a D8 Advance instrument (Bruker AXS) with Ni-filtered Cu Kα radiation (wavelength = 0.154 nm) from 5° to 60°. The glucose content in the enzyme hydrolyzate was analyzed by a high-performance anion exchange chromatography (HPAEC) (Dionex, ICS 3000, U.S.) system equipped with an integral amperometric detector and CarboPac PA 100 (Dionex, U.S.) analytical column.

3. Results and discussion

3.1. Regeneration and drying of cellulose from BMIMCl through different methods

As we mentioned above, during the regeneration process, recrystallization of cellulose was hardly avoided (Lozano et al., 2012; Zhang et al., 2005). However, because the polarity of acetone was much lower than that of water or ethanol, cellulose regenerated from acetone was significantly different from those from water and ethanol. The images of MCC and these regenerated samples were taken and illustrated in Fig. S1. It was found that cellulose regenerated from water or ethanol was gelatinous before drying process, while cellulose regenerated from acetone was powder even without drying. DRC-e and DRC-a were dehydrated and formed hard lump during the drying process. Like many porous materials, the pore structures would collapse during the traditional drying process and partial hydrogen bonds would reform with the removal of the strong polar solvent around cellulose chains under the driving force of surface tension. Therefore, cellulose II structure usually can be observed in these dried regenerated samples from water or alcohol. Additionally, freeze drying was also applied to avoid the recrystallization of cellulose during the drying process and fluffy aerogel-like cellulose could be obtained.

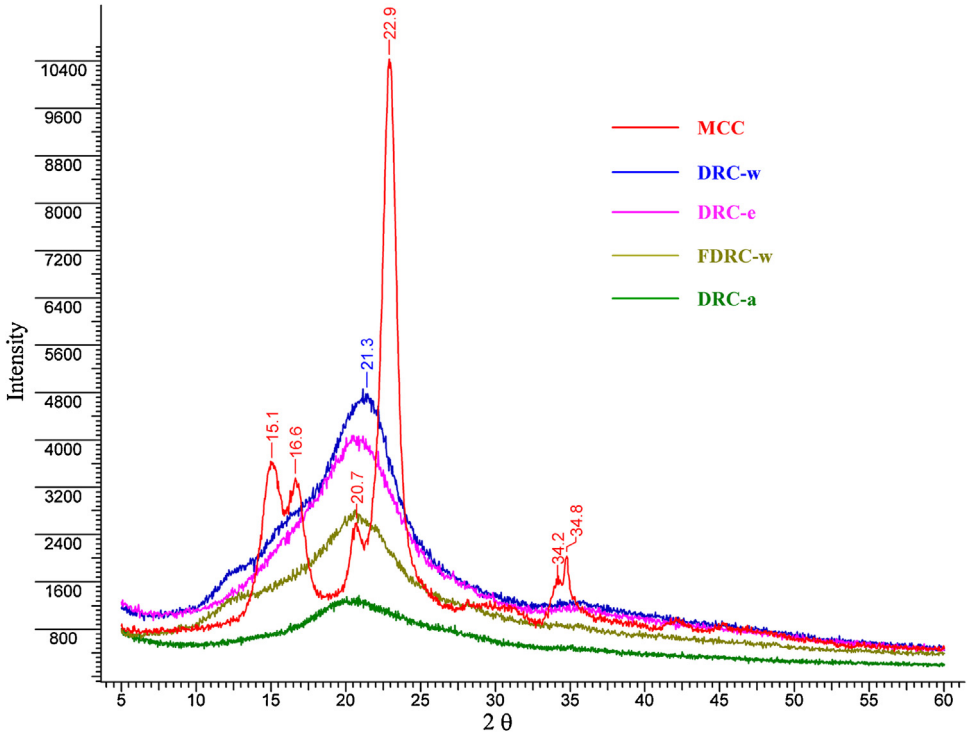

Fig. 1. XRD patterns of MCC and regenerated cellulose samples.

3.2. XRD analysis

In order to exploring the crystalline structure change of MCC during the regeneration and drying processes, XRD patterns of MCC and these regenerated cellulose samples were recorded and illustrated in Fig. 1. It can be seen that significant difference can be found between MCC and regenerated cellulose. The diffraction peak of MCC was much higher than others, indicating the high crystallinity of MCC. The five characteristic peaks observed in MCC suggested a typical pattern of cellulose I (Mathew, Oksman, & Sain, 2005). However, all these regenerated samples only displayed a broad peak at around 21.3° and the intensity followed the order of DRC-w > DRC-e > FDRC-w > DRC-a, suggesting that cellulose II structure was existed in these regenerated samples with varying degrees. Cellulose regenerated from acetone (DRC-a) showed the lowest diffraction intensity among these regenerated cellulose, indicating that acetone was a good solvent for preparing regenerated cellulose with very low crystallinity. Additionally, FDRC-w displayed relatively lower diffraction intensity than DRC-w. It was assumed that during the freeze drying process, water could be directly sublimed from ice to vapor without the route of liquid water and avoid the recrystallization of cellulose to a certain degree (Sathitsuksanoh, Zhu, Wi, & Percival Zhang, 2011).

3.3. Enzymatic hydrolysis

The enzymatic hydrolysis rate and conversion ratio of cellulose were critical parameters to evaluate the enzymatic hydrolysis behavior. As we mentioned above, the crystallinity of cellulose could affect its enzymatic hydrolysis to some degree. Therefore, the enzymatic hydrolysis behaviors of MCC, DRC-w, FDRC-w, DRC-a, and WRC-w were measured and illustrated in Fig. 2. Obvious differences could be observed among these samples. The enzymatic hydrolysis rate increased dramatically with the decrease of cellulose crystallinity. The enzymatic hydrolysis rate was in the order of DRC-a and WRC-w > FDRC-w > DRC-w > MCC. As expected, among these samples WRC-w showed much high enzymatic hydrolysis rate. However, DRC-a regenerated from acetone with the lowest crystallinity displayed a comparative enzymatic hydrolysis rate and conversion ratio to WRC-w, and these values were significantly higher than these of MCC. For WRC-w and DRC-a, a conversion ratio higher than 90% could be achieved within 4 h. Similar results for the enzymatic hydrolysis of wet regenerated cellulose and MCC had been reported in previous studies (Lozano et al., 2012; Shill et al., 2011). Additionally, FDRC-w also showed higher enzymatic hydrolysis rate and conversion ratio than DRC-w, which was well in line with a previous study (Sathitsuksanoh et al., 2011). But due to the relatively high crystallinities of DRC-w and MCC, their enzymatic hydrolysis rates and conversion ratios were not high. The conversion ratios of all regenerated samples after 96 h could reach 85.1–99.4%, which is in sharp contrast with 48.5% of MCC.

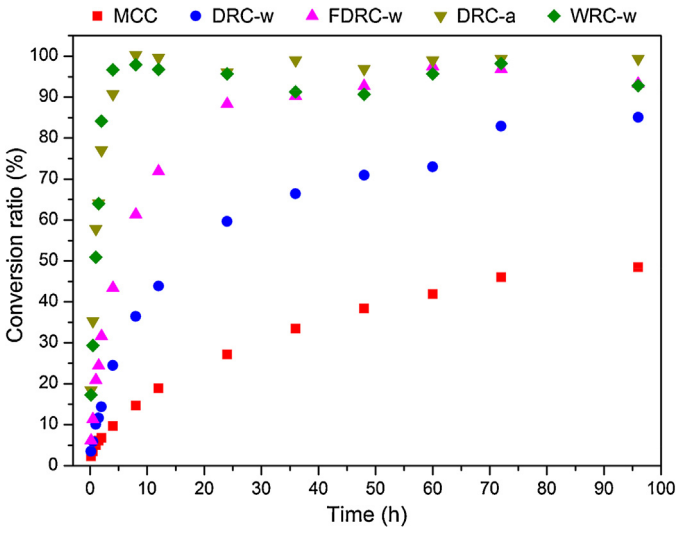

Fig. 2. The enzymatic hydrolysis behaviors of MCC, DRC-w, FDRC-w, DRC-a and WRC-w.

4. Conclusions

Various regenerated cellulose samples were prepared from different solvents and drying methods. XRD results showed that cellulose regenerated from acetone displayed a much lower crystallinity than other samples. The enzymatic hydrolysis results suggested that low crystallinity was contributed to enhance the enzymatic hydrolysis rate and the conversion ratio of cellulose to glucose. For cellulose regenerated from acetone (DRC-a) and wet regenerated cellulose from water (WRC-w), above 90% of cellulose could be converted into glucose within 4 h, which was in sharp contrast with 48.5% of conversion ratio for MCC after 96 h.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.05.004.

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