RSC Advances

PAPER

Cite this: RSC Adv., 2014, 4, 26838

Hydrolysis of crystalline cellulose to glucose in an autoclave containing both gaseous and liquid water⁺

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Hydrolysis of cellulose was investigated in an autoclave. The yield of glucose produced from crystalline cellulose was dependent on the amount of water added to the autoclave. The highest glucose yield of 17% was obtained after 5 h reaction at 190 °C in the presence of a small amount of liquid water in the autoclave. When hydrolysis was carried out in saturated vapor pressure in the autoclave in which cellulose was separated from liquid water, nearly the same yield of 15% was obtained. However, when cellulose was soaked in water, the yield was decreased to about 5%. The results suggested that water vapor plays an important role in hydrolysis of the β -1,4-glycosidic bonds of cellulose. In addition, hydrolysis of the β -1,4-glycosidic bonds and degradation of produced glucose under hydrothermal conditions were investigated by using cellobiose (a dimer of glucose) and authentic glucose, respectively.

Received 19th March 2014 Accepted 9th June 2014 DOI: 10.1039/c4ra02396j

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Introduction

Cellulose is the most abundant source of biomass on earth and is a valuable renewable energy resource.¹ Glucose, which can be obtained by hydrolysis of cellulose, is expected to become one of the main feedstocks for various chemicals and fuels in the future.² However, efficient hydrolysis of cellulose to glucose by an environmentally friendly process remains a challenge because of the robust crystalline structure of cellulose, which is composed of glucose linked by β-1,4-glycosidic bonds and hydrogen bonds between the glucose units. The configuration of the β -1,4-glycosidic bonds in the crystal structure inhibits water molecules and also catalysts from approaching the bonds. This limitation makes hydrolysis of cellulose much slower than that of starches.3 Various approaches have been proposed to circumvent this difficulty. They include reactions using catalysts such as homogeneous mineral acids,⁴ enzymes,⁵ and heteropoly acids,6 reactions in ionic liquids.7 However, these processes have drawbacks including difficulty in separation, high cost, low reaction rate and low glucose selectivity.

Among the various approaches, hydrolysis of cellulose into glucose using solid catalysts has been attracted much attention due to easy separation of the catalysts from reaction solutions, their high reusability, and relatively mild reaction conditions.⁸⁻¹⁰ Considerable efforts have been directed toward the development of catalysts for hydrolysis of cellulose into glucose

or other useful compounds in a closed reaction system. Onda et al. carried out hydrolysis of cellulose over sulfonated activated carbon and obtained glucose in a yield (moles of glucose produced/moles of the glucose units in cellulose) of 41% and selectivity of more than 90% after reaction at 150 °C for 24 h.⁸ Suganuma et al. reported hydrolysis using amorphous carbon bearing SO₃H, COOH, and OH functional groups as catalysts and obtained glucose in a yield of 4% and water soluble β -1,4glucan in a yield of 64% after reaction at 100 °C for 3 h.⁹ Kobayashi et al. reported hydrolysis of cellulose into glucose over mesoporous carbon (CMK-3) catalysts on which transition metals had been deposited. They obtained glucose at the highest yield of 34% with 10 wt%Ru/CMK-3 at 230 °C.¹⁰

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In these reaction systems, ball milling was widely used as a pretreatment of crystalline cellulose to break up the crystalline structure. Once the crystalline structure is destroyed, it becomes easier for reactants and catalysts to access the β -1,4-glycosidic bonds of the glucose units of cellulose. Using cellulose that had been treated by ball milling with preparing K26 carbon powder as a catalyst, Kobayashi *et al.* obtained high conversion of cellulose and high yields of glucose (20% at 180 °C and 57% at 230 °C) under hydrothermal conditions without mineral acid. They attributed the effect of ball milling to good physical contact between cellulose and the carbon catalyst.¹¹ Although ball milling is an easy process, it takes a long time and large mechanical energy to significantly decrease the crystallinity of cellulose, ¹² which is a serious drawback for utilization of natural resources in a large scale by environmentally friendly methods.

Concerning chemicals and solvents, pure water is the most ideal solvent and reactant for hydrolysis of cellulose because water is the most environmentally friendly solvent and is able to dissolve low-molecular-weight products, such as glucose,

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[†] Electronic supplementary information (ESI) available. See DOI: 10.1039/c4ra02396j

cellobiose and 5-hydroxymethylfurfural at high concentrations. Efficient hydrolysis of crystalline cellulose can be achieved using hot-compressed water in a supercritical state without catalyst, although the process requires severe reaction conditions.¹³ Jollet *et al.* reported 35% conversion of cellulose after processing for 24 h at 190 °C under 5 MPa of H₂, although the yield was very low (2%).¹⁴ In some studies on enzymatic hydrolysis of biomass materials including crystalline cellulose, the materials were treated with water vapor beforehand.¹⁵ The results gave us an idea that water vapor may have a favorable effect on the hydrolysis of crystalline cellulose, in this study, we tried to find conditions to hydrolyze cellulose, especially crystalline cellulose, using only water as the reagent and investigated the role of water vapor in the hydrolysis.

Experimental

A typical reaction procedure for hydrolysis of cellulose was as follows. About 324 mg of crystalline cellulose (MERCK, microcrystalline) was dispersed into a desired amount of distilled water (0.3-10 mL). The mixture was transferred into a 100 mL Teflon-lined steel autoclave. The autoclave was sealed and then placed in a hydrothermal synthesis reactor (Hiro Co., Japan) and attached to the rotary drive shaft in the heater unit. The reaction was carried out at 160 and 190 °C for several hours with rotation at 20 rpm. After the reaction process, the autoclave was removed from the reactor and cooled to room temperature. The reaction solution was transferred into a sample tube and 15 mL of distilled water was added to the reaction solution. Then the mixture was separated to solid and liquid parts by centrifugation. The solid part was dried and weighed, and a portion of the liquid part was sampled with a syringe and subjected to liquid chromatographic analysis using a Hitachi HPLC (with a Rezex RPM-Monosaccharide Pb + 2 (8%) column) equipped with a refractive index detector. Ball-milling treatment was conducted at room temperature to decrease the crystallinity of cellulose. Cellulose powder (20 g) was put into an alumina bottle containing alumina balls and the bottle was rotated for 7 days at 400 rpm. XRD patterns were recorded using a Rigaku MiniFlex X-ray diffractometer (CuKa, Ni filter).

Results and discussion

Although the target of our present study is hydrolysis of crystalline cellulose, we also studied hydrolysis of amorphous cellulose as the reference because amorphous cellulose is frequently used in this field.^{8,10,11} Typical XRD patterns of cellulose before and after ball-milling treatment are shown in Fig. 1: the peaks at $2\theta = 16^{\circ}$, 22° and 34° are assigned to (101), (002) and (040) facets, respectively. These peaks became weaker and broader with processing time of ball milling. They were hardly observed after processing for 7 days, indicating that the crystalline phase was all converted to the amorphous phase. The cellulose without the processing and after treatment for 7 days are hereafter called crystalline cellulose and amorphous cellulose, respectively.



Fig. 1 XRD patterns of cellulose (a) before the ball-milling treatment, (b) after the treatment for 3 days, and (c) after the treatment for 7 days.

Hydrolysis of crystalline cellulose and amorphous cellulose was carried out at 160 and 190 °C for 5 h in an autoclave containing 5 mL of water. Since the expected amounts of water vaporized in the autoclave at 160 and 190 °C were about 16.6 mmol (0.32 mL) and 33.3 mmol (0.62 mL), respectively, as discussed later, cellulose was soaked in liquid water in the autoclave during hydrolysis. The yields of glucose obtained from crystalline cellulose were less than 0.2% at 160 °C and less than 11.0% at 190 °C. However, when amorphous cellulose was used, the yields of glucose were increased to 7.8% and 33.5% at temperatures of 160 and 190 °C, respectively. The favorable effect by amorphousization is consistent with the report by Zhao et al.12 Although hydrolysis of cellulose can be accelerated by amorphousization, activation by the process of ball milling does not seem to be a good solution due to its long process time and the mechanical energy needed.

To find conditions to increase the yield of glucose from crystalline cellulose, we simply changed the amount of water added to the autoclave. Fig. 2 shows the results for hydrolysis of crystalline cellulose carried out at 160 °C and 190 °C for 5 h in the autoclave with increase in the amount of water added. Interestingly, the yield showed strong dependence on the amount of water added. The glucose yields reached the maxima when about 2 mL of water was added to the autoclave: the maximal yields were 0.2% and 17% at 160 °C and 190 °C, respectively. Cellobiose, a dimer of glucose linked by a β -1,4-glycosidic bond, and 5-hydroxymethylfurfural (5-HMF), a decomposed product from glucose, were the major by-products of the reaction solution. The yields of cellobiose and 5-HMF were 0.3% and 1.6%, respectively, after reaction for 5 h at 190 °C in an autoclave containing 2 mL of water.

The amount of vaporized water in the autoclave can be estimated using Teten's equation, which correlates saturated vapor pressure $P_{sat}(T)$ at a temperature (*T*) by

$$P_{\rm sat}(T) = 0.611 \times 10^{7.5T/(T+273.3)},\tag{1}$$



Fig. 2 Yield of glucose produced by hydrolysis of crystalline cellulose as a function of water added to the autoclave (100 mL). The hydrolysis was carried out for 5 h at (a) 160 °C and (b) 190 °C. The plots for (a) are enlarged by 20 times. Cellulose: 324 mg, water: 2 mL. It was estimated from eqn (1) that water was fully vaporized in the autoclave when the amounts of water added were less than 0.32 mL and 0.62 mL for cases (a) and (b), respectively. When the amounts of water added were larger than these critical values, the exceeding parts remained as liquid.

where *T* is given in $^{\circ}$ C and vapor pressure is in kPa.¹⁶ Using this equation, the amounts of water vaporized in the Teflon-lined tube (100 mL) at 160 $^{\circ}$ C and 190 $^{\circ}$ C were estimated to be 16.6 mmol (0.32 mL) and 33.3 mmol (0.62 mL), respectively.

The yield of glucose was decreased when the amount of water added was less than 0.6 mL, as shown in Fig. 2. This is probably because all of the water is vaporized and the pressure of water in the system becomes low with decrease in the amount of water added. The yield of glucose was also decreased with an increase in the amount of water added when the amount was more than 2 mL. When amorphous cellulose was used instead of crystalline cellulose, decrease in the yield of glucose with increase in the amount of water (more than 2 mL) was not observed (see Fig. S1 in ESI[†]).

It is thought that crystalline cellulose has specific interactions with liquid or vaporized water. More specifically, the fact that the maximum yield of glucose was obtained by adding 2 mL of water (Fig. 2) suggests that too much liquid water has a harmful effect on the hydrolysis, which will be discussed later. This fact also suggests that a small amount of liquid water is effective for enhancing the yield because the highest yields of glucose were obtained when a small amount of liquid water was expected to be present in the autoclave. A small amount of liquid water may be useful for removing polymerized products from the surface of cellulose fibers.

The harmful effect of a large amount of liquid water on the production of glucose suggests that hydrolysis of crystalline cellulose is carried out mostly by water vapor and that liquid water prevents contact of β -1,4-glycosidic bonds with water vapor. In the case of liquid water, hydrolysis becomes difficult because the intermolecular hydrogen bonds between cellulose molecules and hydrogen bonds with water are strengthened.^{3,17}

Hence, the highest yields of glucose are expected to be obtained under conditions in which a large number of gaseous water molecules can make access to the β -1,4-glycosidic bonds of

cellulose.

Fig. 3 shows the time course of conversion of crystalline cellulose and yield of glucose when the reactions were carried out at 160 °C (Fig. 3a) and 190 °C (Fig. 3b) by addition of 2 mL of water, at which the yields of glucose showed the maxima (Fig. 2). In the case of reaction at 160 °C, glucose yield reached the highest value of 16% at 45 h and the conversion reached 40% at about 50 h. When the reaction was carried out at 190 °C, the reaction rate was enhanced and it took less than 7 h for the conversion to reach 40%. The conversion increased monotonously with reaction time, reaching about 60% at 10 h. The yield of glucose at 190 °C reached 22% at 8 h and then leveled off or started to decrease gradually. Leveling off of the yield of glucose was also observed for the reaction at 160 °C as shown in Fig. 3a. The leveling off of the yield or the decrease in the yield of glucose is probably due to the degradation and/or polymerization of glucose produced. With regard to cellobiose, its vield reached 0.3% after reaction for 6 h at 190 °C and then started to decrease gradually. In contrast, the yield of 5-HMF increased monotonously with reaction time and reached 3.3% after reaction for 10 h at 190 °C (see Fig. S2†). This monotonous increase of 5-HMF suggests that it is produced via glucose. To confirm the degradation of glucose, we put reagent-grade glucose into the Teflon-lined tube and heated it at 160 and 190 °C in the autoclave containing 2 mL of water. The results showed that glucose is gradually decomposed/polymerized under the conditions of hydrolysis of cellulose, as shown in Fig. 4. Polymerization of glucose or the products from it was thought to have occurred because the residue and solution after the reaction were strongly colored. It is worth noting that the reaction rate was independent of the amount of water added to the autoclave as long as the amount was larger than 1 mL; under that condition, glucose was dissolved in liquid water.

In order to obtain information about hydrolysis of the β -1,4-glycosidic bond of cellulose, we investigated hydrolysis of cellobiose in the autoclave containing 2 mL of water. The initial



Fig. 3 Yield of glucose and conversion of cellulose by hydrolysis of crystalline cellulose at (a) 160 $^\circ$ C and (b) 190 $^\circ$ C. Cellulose: 324 mg, water: 2 mL.



Fig. 4 Degradation of glucose at (a) 160 $^\circ\text{C}$ and (b) 190 $^\circ\text{C}.$ Glucose: 324 mg, water: 2 mL.

production rate of glucose from cellobiose was much higher at 190 °C than at 160 °C, as shown in Fig. 5. The apparent induction periods are probably due to the gradual increase in the actual temperature of the autoclave to the set temperature and not due to the essential properties of the reaction. The production of glucose from cellobiose is more efficient than that from crystalline cellulose probably because water can easily access the β -1,4-glycosidic bond of cellobiose. At 190 °C, the yield of glucose started to decrease after 3 h reaction due to the degradation of glucose.

The effect of water vapor and liquid water on crystallinity of cellulose was investigated. XRD patterns of cellulose after processing at 190 °C for 4 h and 8 h in the autoclave containing 2 mL of water were almost the same as the XRD pattern of original cellulose (see Fig. S3†). During the processing, some of the cellulose was converted to glucose. This result suggests that the crystallinity of cellulose was not affected by the processing and that the hydrolysis took place at the surface of crystalline cellulose fibers. Similar results have been reported by Xiao *et al.*¹⁸

Fig. 6 shows the amount and yield of glucose produced from crystalline cellulose as a function of the amount of cellulose added to the autoclave that contained 2 mL of water. The reaction was carried out at 190 °C for 4 h. The amount of glucose produced increased with increase in the amount of cellulose added, which is attributed to the increased surface area of crystalline cellulose. However, the yield of glucose was low when the amount of cellulose was small (less than 100 mg). The small amount of cellulose was probably completely soaked in liquid water and had less chance to be exposed to water vapor; the amount of liquid water was estimated to be about 1 mL, as stated above. Hence, this result also suggests the importance of water vapor for hydrolysis of crystalline cellulose.

Here a question arises as to whether hydrolysis of crystalline cellulose proceeds in the absence of liquid water, if water vapor is supplied to the surface of the cellulose fiber. To answer the question, hydrolysis of crystalline cellulose was carried out at 190 °C by exposing cellulose only to water vapor in the autoclave. In the experiment, cellulose was put in a small beaker placed away from liquid water inside the autoclave, which was not rotated. As a result, we obtained glucose in a yield of about 15%, which was nearly the same as the highest yield obtained (about 17%) by using 2 mL of water, as shown in Fig. 2. In this arrangement, naturally, the amount of liquid water added hardly affected the yield of glucose, if the amount was large enough to obtain saturated vapor pressure, as shown by the broken line in Fig. 7. This tendency is in contrast to the case in which cellulose was not separated from liquid water in the autoclave, which was not rotated, as shown by the dotted line; the yield of glucose started to decrease sharply when the amount of water exceeded 1 mL. For reference, the result obtained without separating cellulose from liquid water is shown by the solid line, which is the same as Fig. 2b; the autoclave was rotated. In this case, the rotation may have enhanced the contact between cellulose and water vapor as long as the amount of liquid water added was not large (or less than 10 mL).



Fig. 5 Hydrolysis of cellobiose at (a) 160 $^{\circ}$ C and (b) 190 $^{\circ}$ C. Cellobiose: 324 mg, water: 2 mL.



Fig. 6 Amount and yield of glucose produced from crystalline cellulose as a function of crystalline cellulose added to the autoclave by hydrolysis for 4 h at 190 °C. Water: 2 mL.



Fig. 7 Yield of glucose produced by hydrolysis of crystalline cellulose as a function of water added. The hydrolysis was carried out at for 5 h at 190 °C under the following conditions; (a) cellulose was separated from liquid water, (b) cellulose was in contact with liquid water in the rotated autoclave (the same results as that shown by the (b) line in Fig. 2), and (c) cellulose was in contact with liquid water in the nonrotated autoclave. Cellulose: 324 mg. It was estimated from eqn (1) that water was fully vaporized in the autoclave when the amounts of water added were less than 0.32 mL and 0.62 mL for cases (a) and (b), respectively. When the amounts of water added were larger than these critical values, the exceeding parts remained as liquid. The states of autoclave under typical conditions are schematically shown in the figure.

All of the results suggest the importance of water vapor for hydrolysis of crystalline cellulose. It should also be noted that liquid water can hydrolyze crystalline cellulose. The yield of glucose was about 4.8%, which was obtained by soaking crystalline cellulose in a sufficient amount of liquid water (10 mL).

Conclusions

Hydrolysis of crystalline cellulose to glucose is generally difficult because of the robust crystalline structure of cellulose. We investigated the hydrolysis under hydrothermal conditions at 160 and 190 °C and found that water vapor plays an important role for the hydrolysis. High glucose yield was obtained when the amount of water added to the autoclave was slightly larger than the amount needed to obtain saturated vapor pressure. Even when cellulose was separated from liquid water, almost the same yield was obtained if the water vapor pressure was saturated.

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