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Rapid Recovery of Fermentable Sugars for Biofuel Production from Enzymatic Hydrolysis of Microcrystalline Cellulose by Hot-Compressed Water Pretreatment

Wenbing Zhou,[†,‡] Yun Yu,[†] Dawei Liu,[†] and Hongwei Wu*[,†]

[†]Department of Chemical Engineering, Curtin University, GPO Box U1987, Perth WA 6845, Australia

[‡]College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, China

ABSTRACT: Enzymatic hydrolysis of microcrystalline cellulose is a multistep heterogeneous reaction limited by the initial action of enzyme to produce short glucose chains, due to the presence of strong intermolecular and intramolecular hydrogen bonding networks in cellulose chains. The results in this study show that enzymatic hydrolysis of the liquid product from hot-compressed water (HCW) pretreatment of microcrystalline cellulose can be immediately converted into glucose oligomers with DPs up to 5 without incubation even at a low enzyme loading (i.e., ~8.6 FPU/g glucan-equivalent). A high enzyme loading (i.e., ~140 FPU/g glucan-equivalent) is able to convert all the glucose oligomers into glucose and cellobiose after 1 h incubation. Overall, the sugar recovery after HCW pretreatment can be drastically increased by up to 2 orders of magnitude, depending on enzyme loading and incubation time. Therefore, HCW pretreatment of microcrystalline cellulose is an effective pretreatment method to break hydrogen bonding networks and convert crystalline bundles of long cellulose chains into soluble glucose oligomers with a wide range of degrees of polymerization (DPs), drastically increasing the chain ends accessibility and enabling enzymatic hydrolysis to take place homogeneously.

1. INTRODUCTION

Lignocellulosic biomass is a promising feedstock for producing second-generation biofuels.[1,2] However, converting lignocellulosic biomass into fermentable sugars is a challenging task due to the complex plant cell structure of these materials. In lignocellulosic biomass, cellulose is closely associated with hemicellulose and lignin, while lignin is also naturally resistant to chemical or biological attack due to its covalent bonds to hemicellulose and cross-linking to cellulose microfibrils.[3,4] Therefore, pretreatment of lignocellulose biomass is essential to disrupting the cell wall structure of lignocellulosic cellulose, e.g., remove lignin and hemicellulose and enhance the accessibility of cellulose.[3,5] Effective pretreatment can dramatically reduce enzyme loading and hydrolysis reaction time hence significantly reduce the production cost and the hydrolysis reactor footprint.[6,7] Currently, pretreatment is one of the most expensive steps during biochemical conversion of lignocellulosic biomass to ethanol.[8,9] Among various pretreatment methods,[10] hot-compressed water (HCW) pretreatment is of low-cost due to the elimination of chemical use and reduction in capital cost.[11] Substantial works were carried out to pretreat lignocellulosic biomass by HCW for subsequent enzymatic hydrolysis process.[12−18] However, the main development so far mainly focuses on the use of HCW pretreatment to remove hemicellulose and lignin under mild conditions, with the objective of producing a cellulose-rich residue that is used for subsequent enzymatic hydrolysis. Such a pretreatment increases the accessibility of cellulose from lignocellulosic biomass via effective fractionation (i.e., largely removing hemicellulose and lignin). However, the overall reaction rate of enzymatic hydrolysis of the residue cellulose is still low, typically requiring up to several days to complete the saccharification step.[10] Therefore, it is highly desired to further drastically reduce the hydrolysis time (e.g., from days to hours or even shorter) in order to significantly improve the economic performance of cellulosic second-generation biofuel production.

Cellulose produced from biomass pretreatment generally has crystalline structures due to strong intermolecular and intramolecular hydrogen bonding networks existing between cellulose chains.[19] Enzymatic hydrolysis of cellulose follows multistep heterogeneous reactions where glucose is produced via the synergistic actions of three types of cellulases.[20] For example, endoglucanases (EC 3.2.1.4) randomly break the β-1,4-glycosidic bonds on cellulose chains away from chain ends, cellobiohydrolases (or exoglucanases, EC 3.2.1.91) produce cellobiose by attacking cellulose from chain ends, and β-glucosidases (EC 3.2.1.21) produce glucose from cellobiose or other short cellooligosaccharides.[20] One important reason for the slow reaction rate during enzymatic hydrolysis of microcrystalline cellulose is that such material has very limited chain ends, thus making the initial reactions, i.e., to produce short glucose chains by cellobiohydrolases, difficult to take place.[20] Therefore, to achieve a high hydrolysis rate, it is essential to destructing the intermolecular and intramolecular hydrogen bonding networks within crystalline cellulose in order to release more chain ends. For example, it was recently reported that ionic liquid pretreatment alters cellulose structure via converting inert cellulose I to reactive cellulose II, which is highly susceptible to enzymatic hydrolysis.[21,22] By pretreating lignocellulosic biomass with ionic liquid at 120 °C, complete hydrolysis of cellulose into glucose was achieved within 24 h.[21] Other methods have also been proposed, such as that deploys a

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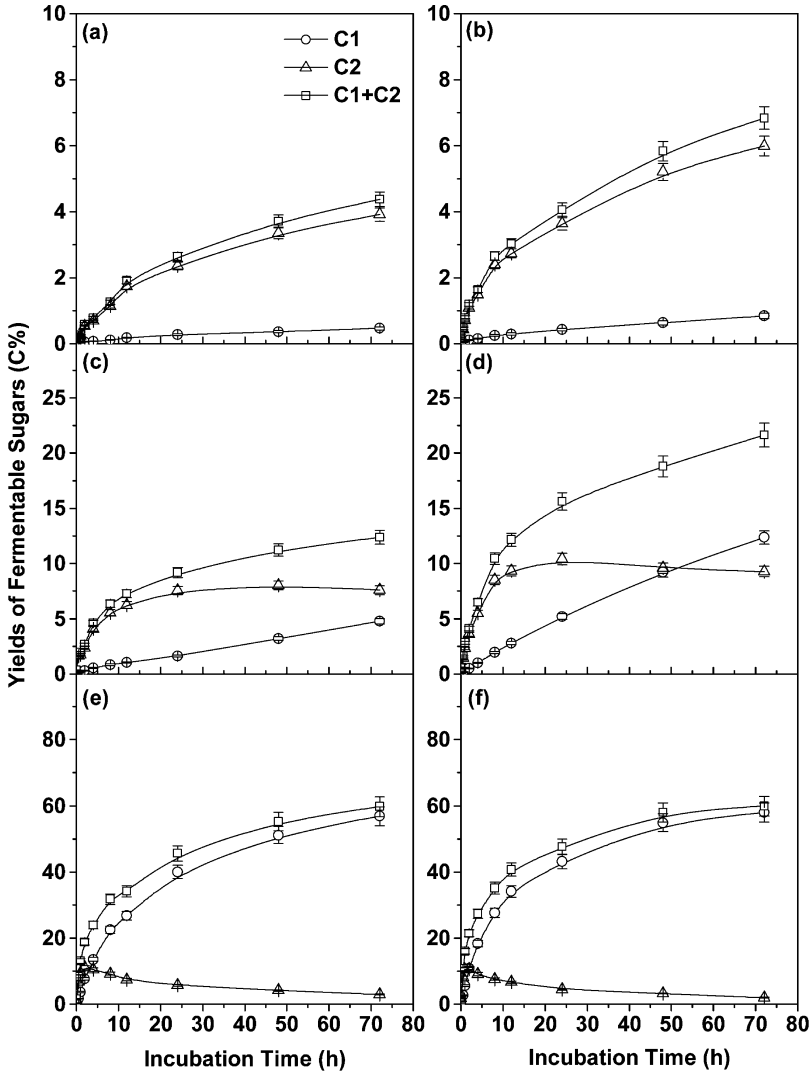

Figure 1. Yields of fermentable sugars from enzymatic hydrolysis of microcrystalline cellulose at various enzyme loadings: (a) 0.7 FPU/g glucan; (b) 1.4 FPU/g glucan; (c) 4.3 FPU/g glucan; (d) 8.6 FPU/g glucan; (e) 86 FPU/g glucan; (f) 140 FPU/g glucan. Note: yields are based on the total hydrolyzable sugars in the substrate.

sequential combination of organic acid and base.[23] However, such methods suffer from the issues of recycling chemicals such as ionic liquid[24] and/or also potential toxicity.[25]

HCW pretreatment eliminates the use of expensive chemicals,[26−28] with the cleavage of hydrogen bonds in crystalline cellulose occurred at 180 °C or above.[29] However, the typical pretreatment temperatures (180−230 °C) which are suitable for removing hemicellulose and lignin from biomass are not sufficiently high for rapidly breaking the hydrogen bonds in crystalline cellulose.[17] Higher temperatures are required to increase the reaction rate of converting cellulose to short-chain glucose oligomers in HCW. However, sugar decomposition issue arises as temperature increases,[30,31] leading to the formation of possible inhibitors for enzymatic hydrolysis and subsequent ethanol fermentation.[32] Therefore, it is important to minimize the sugar decomposition at increased temperatures to achieve a high sugar recovery. Recent work by this group has confirmed that the decomposition reaction of sugar can be minimized using a semicontinuous reactor under optimized conditions.[28,33,34] Via HCW pretreatment at 280 °C, microcrystalline cellulose can be completely converted into glucose oligomers with a wide range of degrees of polymerization (DPs) of 1−30, with over 80% (on a carbon basis) of total sugar recovery in the forms of short-chain glucose oligomers.[28] Therefore, it is envisaged that HCW pretreatment will liberate a substantial number of chain ends, hence eliminating chain end availability as a limiting factor for enzymatic hydrolysis. The objective of this study is to demonstrate that drastic improvement in hydrolysis rate (and therefore reduction in hydrolysis reaction time) can be achieved if liquid products produced from HCW pretreatment of microcrystalline cellulose in a semicontinuous reactor, instead of microcrystalline cellulose itself, are used for fermentable sugar recovery via enzymatic hydrolysis.

2. EXPERIMENTAL SECTION

2.1. Materials. Microcrystalline cellulose (Avicel PH-101) and cellulase (*Trichoderma reesei* ATCC 26921) were purchased from Sigma-Aldrich. The activity of celluase was measured based on the standard filter paper assay detailed elsewhere.[35] Glucose oligomer standards with DPs up to five (i.e., glucose, cellobiose, cellotriose, cellotetraose, and cellopentaose), and high-purity reagents also were also purchased from Sigma-Aldrich.

2.2. HCW Pretreatment of Microcrystalline Cellulose. HCW pretreatment of microcrystalline cellulose was carried out using a

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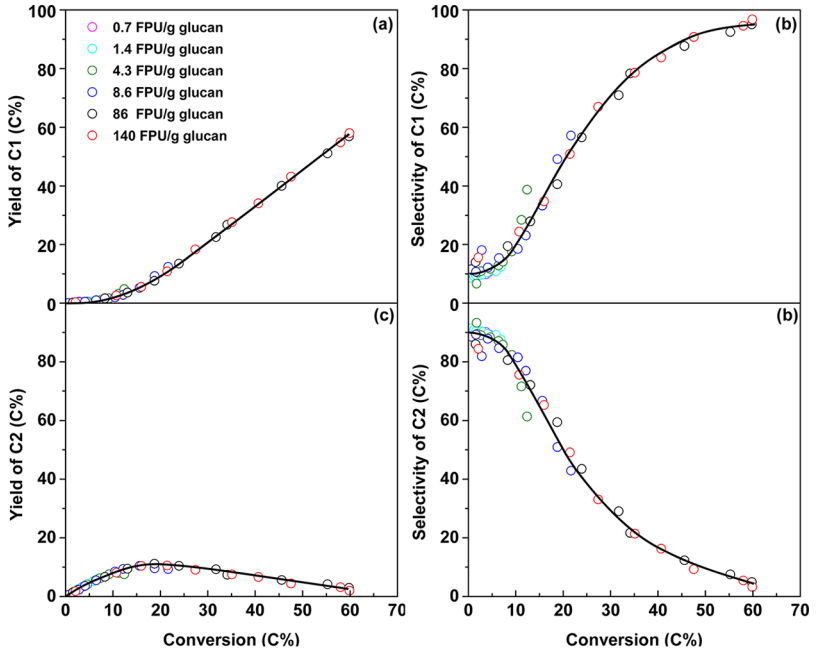

Figure 2. Yields and selectivities of glucose and cellobiose as a function of cellulose conversion from enzymatic hydrolysis of microcrystalline cellulose at various enzyme loadings: (a) yield of glucose; (b) selectivity of glucose; (c) yield of cellobiose; (d) selectivity of cellobiose.

semicontinuous reactor system that is similar to the one used in our previous studies.[28,29,33,34,36] The detailed description of the reactor system can be found elsewhere.[34] A unique feature of the reactor system is that the liquid product is rapidly swept out of the reactor system and quenched by an ice water bath, thus minimizing the secondary decomposition reactions of sugar products. Briefly, ~50 mg of microcrystalline cellulose was sandwiched in a reactor cell. The reactor system was housed in an infrared gold image furnace. Before heating, deionized water flew through the reactor system at a flow rate of 10 mL min^{-1} via an HPLC pump. The reactor cell was then rapidly heated to 280 °C within 2 min and further held at the reaction temperature for 20 min for complete conversion. The liquid product was quickly quenched and collected for subsequent enzymatic hydrolysis experiments. The reaction pressure was maintained at 10 MPa using a back-pressure regulator.

2.3. Enzymatic Hydrolysis Experiments. For enzymatic hydrolysis of microcrystalline cellulose, ~50 mg microcrystalline cellulose was added into 50 mL of sodium acetate buffer solution (pH = 4.8) at various enzyme loadings of 0.7−140 FPU/g substrate (glucan equivalent) and then incubated in a water bath preheated to 50 °C. Liquid products were sampled once desired incubation times (0−72 h) were reached and immediately immersed in a hot water bath at ~100 °C for 10 min to stop the enzymatic activity. The samples were then stored in a freezer at 0 °C for further analysis. For enzymatic hydrolysis of liquid products after HCW pretreatment, a similar procedure was followed with an enzyme loading of 1.4−140 FPU/g substrate (glucan equivalent). The cellulase enzymes added into the solutions were calculated based on the glucan equivalent − the total hydrolyzable sugar determined by dilute acid hydrolysis at 121 °C for 1 h, following a NREL method.[37] Comparison between the results from the hydrolysis of microcrystalline cellulose and the liquid product from its HCW pretreatment is based on the glucose produced as a percentage of the theoretical yield of monomeric sugar (i.e., total acid-hydrolyzable sugars).

2.4. Quantification of Sugars in Liquid Samples. The fresh liquid products were immediately analyzed after collection from HCW pretreatment, since the glucose oligomers with high DPs easily precipitate from liquid sample.[34] The total carbon content was quantified by a total organic carbon (TOC) analyzer (Shimadzu Model TOC-V_{CPH}). The distribution of glucose oligomers in liquid samples including those after HCW pretreatment and those after enzymatic hydrolysis was analyzed by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) using a Dionex ICS-3000 ion chromatography (IC) system. The detailed procedures for IC analysis can be found elsewhere.[34] The glucose oligomers with DPs up to 5 were quantified using available standards. In this paper, the glucose oligomers are named based on DP values (e.g., glucose as C1, cellobiose as C2). All experiments were performed in triplicates, and error bars show standard deviations.

3. RESULTS AND DISCUSSION

3.1. Enzymatic Hydrolysis of Microcrystalline Cellulose. Enzymatic hydrolysis of microcrystalline cellulose was carried out at various enzyme loadings from low (0.7 FPU/g glucan) to high (140 FPU/g glucan) in order to determine a suitable enzyme loading at which the availability of enzyme is not a limiting factor for cellulose hydrolysis. It is noted that during the course of enzymatic hydrolysis, glucose and cellobiose were the only two enzymatic hydrolysis products identified in the liquid product. It was expected that glucose oligomers would account for a large fraction of the intermediate products from enzymatic hydrolysis of cellulose, but no glucose oligomers with various DPs were detected in the liquid products collected from the enzymatic hydrolysis of cellulose. The yields of glucose and cellobiose from enzymatic hydrolysis of cellulose at various enzyme loadings are calculated on a carbon basis. The results are presented in Figure 1, which shows that the total yield of glucose and cellobiose increases with enzyme loading. For example, the total yield of glucose and cellobiose after 1 h incubation increases from ~0.3% at 0.7 FPU/g glucan to ~13% at 86 FPU/g glucan. After 24 h incubation, the total yield increases from ~2.6% to ~46%, when the enzyme loading increases from 0.7 to 86 FPU/g glucan. However, the total yield of glucose and cellobiose remains almost unchanged as the enzyme loading further increases from 86 to 140 FPU/g glucan. These results indicate that the yields of glucose and cellobiose are indeed limited by the availability of cellulase enzymes, especially at low enzyme

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loadings (e.g., 8.6 FPU/g glucan or lower). When the enzyme loading increases to ~86 FPU/g glucan, the availability of enzyme is no longer a limiting factor for enzymatic hydrolysis of cellulose.

The selectivities of glucose and cellobiose at different enzyme loadings were further calculated, and the yields and selectivities were plotted as a function of cellulose conversion in Figure 2. The results show that at various enzyme loadings, all the data fit into similar curves in terms of selectivities and yields, suggesting that an increase in the enzyme loading leads to little alteration in reaction mechanism of enzymatic hydrolysis although it does substantially accelerate the hydrolysis reaction as shown in Figure 1. The formations of glucose and cellobiose are found to change with cellulose conversion only and the selectivity of glucose continuously increasing with cellulose conversion. Another interesting finding is that cellobiose is the dominant primary product from enzymatic hydrolysis of cellulose, since its selectivity is ~90% (on a carbon basis) at low conversion levels (up to 5%). A small amount of glucose (i.e., ~10% on a carbon basis) is also produced from primary reactions. The results also suggest that cellobiose can be easily converted into glucose, and the yield of cellobiose is below 15% at all conversion levels during enzymatic hydrolysis.

3.2. Enzymatic Hydrolysis of Liquid Products from HCW Pretreatment of Microcrystalline Cellulose. Microcrystalline cellulose was treated by HCW at 280 °C to convert microcrystalline cellulose into a liquid solution which contains mainly short-chain glucose oligomers. The DP distribution of glucose oligomer in the liquid sample was analyzed, and the IC chromatography was similar to that published elsewhere,[34] showing that the liquid sample contains the glucose oligomers with various DPs up to 30. The contents of low-DP glucose oligomers (DP ≤ 5) were quantified with standards, and the content of total hydrolyzable sugar was measured according to dilute acid hydrolysis. As shown in Table 1, ~84% (carbon basis) of liquid products was converted into glucose during post dilute acid hydrolysis. The sugars in the liquid contain mainly high-DP glucose oligomers (DP > 5) or their derivatives, since the total yield of low-DP glucose oligomers (DP ≤ 5) is only ~14%. It is arguable that reducing the temperature of HCW pretreatment may increase the yield of low-DP glucose oligomers; however, it required much longer pretreatment time to achieve complete conversion of microcrystalline cellulose in HCW.[28]

Table 1. Contents of Glucose, Cellobiose, Cellotriose, Cellotetraose, Cellopentaose, and Cellooligosaccharides in the Liquid Products from HCW Pretreatment of Cellulose at 280 °C for 20 min

sugar	content (C%)
glucose (DP = 1)	3.5
cellobiose (DP = 2)	3.0
cellotriose (DP = 3)	2.9
cellotetraose (DP = 4)	2.4
cellopentaose (DP = 5)	1.8
cellooligosaccharides (DP > 5)	70.5
total hydrolyzable sugars	**84.1**

The liquid sample from HCW pretreatment of microcrystalline cellulose was incubated with cellulase enzymes at various enzyme loadings of 1.4−140 FPU/g glucan-equivalent. The yields of low-DP glucose oligomers (DP ≤ 5) were quantified to understand the conversion of high-DP to low-DP glucose oligomers during enzymatic hydrolysis, and the results are shown in Figure 3. It should be noted that the yields are calculated by normalizing the sugars in the product to the total acid-hydrolyzable sugars in the reactant on a carbon basis. The data in Figure 3 demonstrate that the high-DP glucose oligomers (DP > 5) were rapidly converted into low-DP glucose oligomers (DP ≤ 5). For example, at a very low enzyme loading of 1.4 FPU/g glucan-equivalent, all the high-DP glucose oligomers (DP > 5) disappeared within 1 h incubation time and were converted into low-DP glucose oligomers with a total yield of C1−C5 over 80% on a carbon basis. The incubation time required to convert high-DP to low-DP glucose oligomers reduces with increasing enzyme loading. When the enzyme loading increases to 8.6 FPU/g glucan-equivalent, the high-DP glucose oligomers can be immediately converted into low-DP glucose oligomers even without incubation. These results clearly indicate that HCW pretreatment is effective to reduce the reaction time required to convert high-DP to low-DP glucose oligomers during enzymatic hydrolysis. This is of practical importance because such a process is known to be the rate-limiting step in a traditional enzymatic hydrolysis process.[20]

Although the high-DP glucose oligomers are quickly converted into low-DP glucose oligomers after HCW pretreatment, the glucose formation does not seem to increase significantly. As shown in Figure 3, the glucose yield becomes stabilized at low enzyme loadings (≤8.6 FPU/g glucan-equivalent), and the cellobiose yield only initially increases and stabilizes at longer incubation time. The exact reason for this phenomenon is unknown yet. It seems that the cellulase enzymes were inhibited to convert low-DP oligomers and/or cellobiose into glucose, thus reaching an equilibrium state during enzymatic hydrolysis. The inhibition could be caused by end products and other enzyme−inhibitors (e.g., 5-HMF), as the initial reactant already contains a certain amount of glucose and cellobiose. To improve the glucose yield, a very high enzyme loading of 140 FPU/g glucan-equivalent was used for enzymatic hydrolysis. Indeed, the glucose yield increases rapidly with the increase of incubation time at a high enzyme loading (see Figure 3d). After 24 h incubation, the glucose yield increases to ~55% on a carbon basis.

Effect of enzyme loading on the yields of C1−C5 during enzymatic hydrolysis was further compared in Figure 4. The results show that the yields of C1−C5 depend on not only the enzyme loading but also the incubation time. A higher enzyme loading leads to an increased reaction rate for the conversion of high-DP to low-DP glucose oligomers. Figure 4 also shows that the high-DP glucose oligomers rapidly disappear, suggesting that cellulase enzyme has a higher affinity with glucose oligomers with higher DPs. It is noteworthy that at a high enzyme loading of 140 FPU/g glucan-equivalent, almost all the glucose oligomers in the liquid product from HCW pretreatment of cellulose can be rapidly converted into glucose and cellobiose within 1 h incubation time, achieving a total glucose and cellobiose yield of ~82% on a carbon basis. This yield should be the theoretical yield of glucose when all the cellobiose is converted into glucose. Since the yields are based on total hydrolyzable sugar by dilute acid hydrolysis, the results demonstrate that at least some of the sugar oligomers present in the liquid product are hydrolyzable by dilute acid but cannot be converted into glucose by cellulase enzymes. Such sugar oligomers include those containing anhydro-glucose unit(s) in

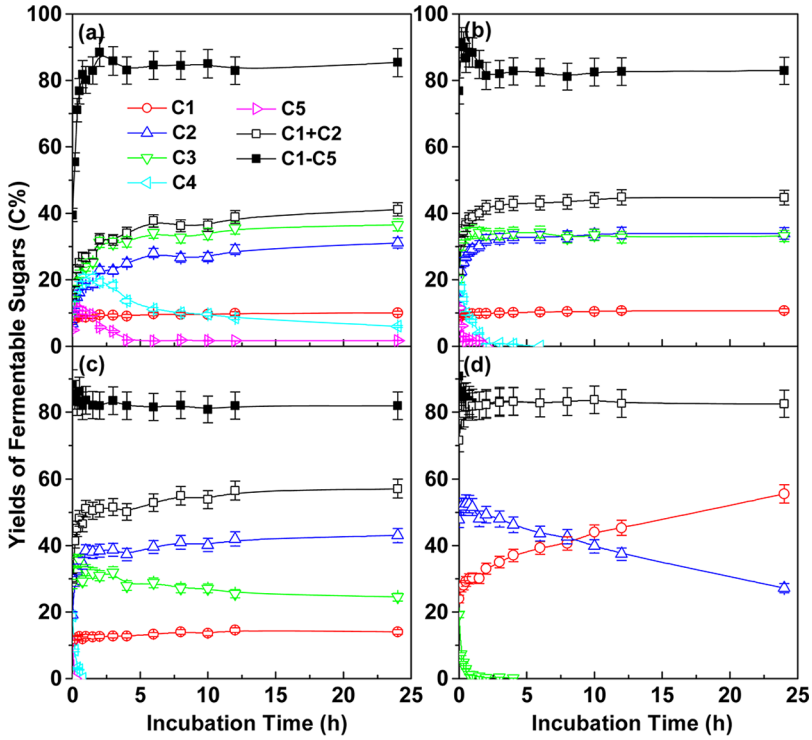

Figure 3. Yields of fermentable sugars from enzymatic hydrolysis of liquid products from HCW pretreatment of microcrystalline cellulose: (a) 1.4 FPU/g glucan-equivalent; (b) 4.3 FPU/g glucan-equivalent; (c) 8.6 FPU/g glucan-equivalent; (d) 140 FPU/g glucan-equivalent. Note: yields are based on the total hydrolyzable sugars in the substrate.

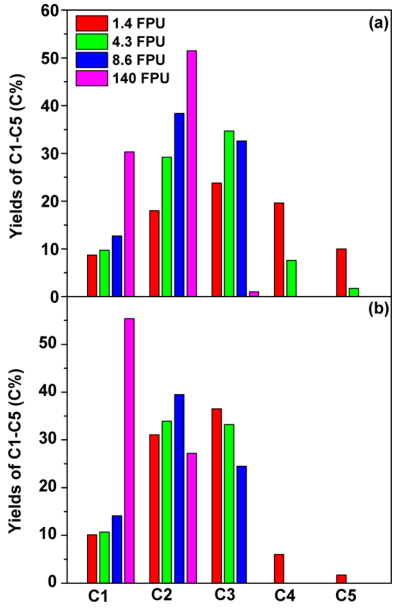

Figure 4. Comparisons of the yields of C1−C5 from enzymatic hydrolysis with HCW pretreatment: (a) after incubation for 1 h; (b) after incubation for 24 h.

the chain, such as levoglucosan and cellobiosan,[38] which can be completely hydrolyzed into glucose by acid but are not completely hydrolyzable by enzymes. The results also indicate that glucose formation is limited by the conversion of cellobiose to glucose, since all the glucose oligomers with DPs > 2 rapidly disappeared within 1 h incubation. Therefore, future work needs to focus on choosing the suitable cellulase enzymes with strong cellobiohydrolase or β-glucosidase reactivities and/or supply additional β-glucosidases during enzymatic hydrolysis of the liquid product produced from the pretreatment of cellulose using HCW.

3.3. Increases in Sugar Recovery from Enzymatic Hydrolysis by HCW Pretreatment. The data so far demonstrate that HCW pretreatment can easily convert microcrystalline cellulose into glucose oligomers with a wide range of DPs, which are considerably more accessible to cellulase enzymes. Since glucose and cellobiose are the main products from direct enzymatic hydrolysis of microcrystalline cellulose, the yields of glucose and cellobiose from enzymatic hydrolysis of cellulose with and without HCW pretreatment were compared at the same enzyme loading. It can be seen in Figure 5 that both the yields of glucose and cellobiose increase drastically with HCW pretreatment. At a low enzyme loading of 1.4 FPU/g glucan-equivalent, the total yield of glucose and cellobiose increases by a factor of 24 after 1 h incubation (see Figure 6), i.e., from 0.8 to 19.5%. In fact, the yield of glucose increases by over 2 orders of magnitude after 1 h incubation, i.e., from 0.08 to 8.7%. As shown in Figure 6, after 24 h incubation, the glucose yield increases from 0.4 to 10.1% after HCW pretreatment, by 23-fold, while the total yield of glucose and cellobiose increases by 1 order of magnitude, from 4.1 to 41.2%. The increases in sugar recovery by HCW pretreatment reduce at an increased enzyme loading, as shown in Figure 6. For example, at an enzyme loading of 8.6 FPU/g glucan-equivalent, the total yield of glucose and cellobiose increases from 2.8 to 51.1% by a factor of 18 after 1 h incubation. For glucose, the yield increases from 0.5 to 12.7% by a factor of 25 after 1 h incubation. After 24 h incubation, the glucose yield increases from 5.2 to 14.1% after HCW pretreatment, only by a factor of 2.7, while the total yield of glucose and cellobiose increases from 15.6 to 53.6% by a factor of 3.4. At a high

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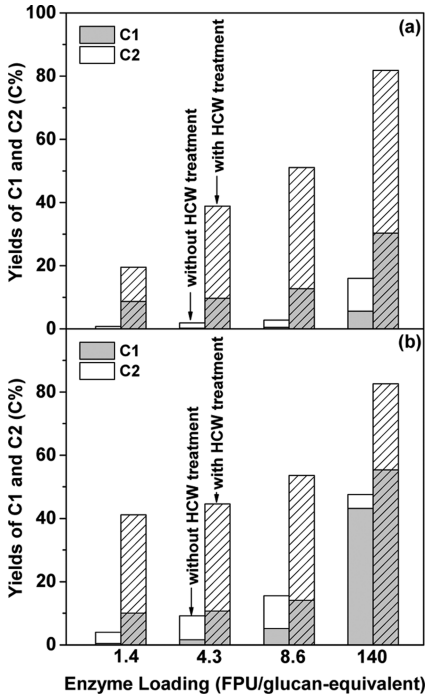

Figure 5. Comparisons of the yields of glucose and cellobiose from enzymatic hydrolysis with and without HCW pretreatment: (a) after incubation for 1 h; (b) after incubation for 24 h. Open: without HCW pretreatment; shade: with HCW pretreatment. Note: yields are based on the total hydrolyzable sugars in the substrate.

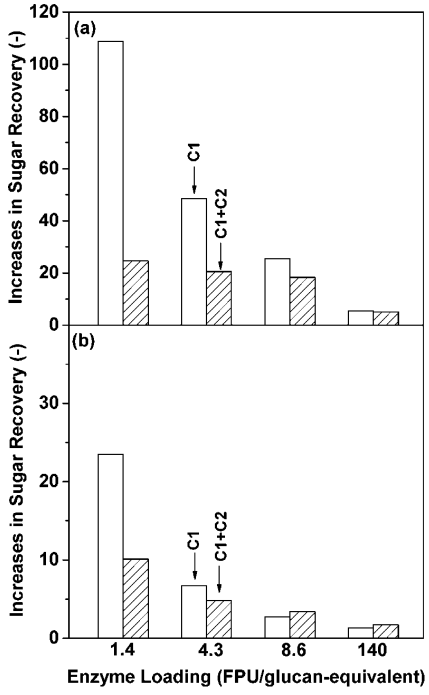

Figure 6. Increases in the sugar recovery from enzymatic hydrolysis by HCW pretreatment: (a) after incubation for 1 h; (b) after incubation for 24 h. Open: increase in glucose recovery; shade: increase in total recovery of glucose and cellobiose.

enzyme loading of 140 FPU/g glucan-equivalent, HCW pretreatment only leads to marginal increases in sugar recovery, i.e., by a factor of 5.4 and 1.3, respectively, for glucose after 1 and 24 h incubation. Therefore, HCW pretreatment is an effective method to substantially increase sugar recovery at low enzyme loadings. This is practically important to reduce the enzyme use during enzymatic hydrolysis. For example, despite a reduction of enzyme use by 2 orders of magnitude, the total sugar recovery at an enzyme loading of 1.4 FPU/g glucan-equivalent with HCW pretreatment is comparable to that at an enzyme loading of 140 FPU/g glucan-equivalent without HCW pretreatment. Clearly, the increases in sugar recovery by HCW pretreatment are due to two reasons. One is that cellulose HCW hydrolysis produces glucose and cellobiose during HCW pretreatment, and the other is that enzymatic hydrolysis of short-chain glucose oligomers can substantially increase their accessibility to cellulase enzymes.

3.4. Implications. The above results demonstrate that HCW pretreatment of cellulose can substantially increase enzymatic hydrolysis reaction rate and reduce enzyme use during the subsequent enzymatic hydrolysis. A schematic diagram is then proposed to illustrate the roles of HCW pretreatment on the subsequent enzymatic hydrolysis as shown in Figure 7. Fundamentally, HCW pretreatment improves the enzymatic hydrolysis via at least two mechanisms. One is that HCW pretreatment of cellulose can effectively convert cellulose into short glucose chains. It is known that the conventional enzymatic hydrolysis is limited by initial reactions to produce short glucose chains, due to the very limited chain ends in microcrystalline cellulose for cellobiohydrolases to attack.[20] HCW pretreatment can effectively destruct the hydrogen bonding networks in crystalline cellulose. The released glucose oligomers provide substantially more chain ends hence the accessibility to cellulase enzymes. The other is that HCW pretreatment converts microcrystalline cellulose into a solution containing various glucose oligomers, enabling enzymatic hydrolysis reaction to take place homogeneously. This significantly increases the enzymatic hydrolysis reaction rate by eliminating enzyme adsorption step that is well-known to be the rate-limiting step in conventional cellulose enzymatic hydrolysis.[39] It should be noted that while HCW pretreatment is effective to convert microcrystalline cellulose into glucose oligomers, there are also possible decomposition of sugars in both the liquid product[28] and solid residue.[33] Therefore, it is important to minimize those sugar degradation reactions by innovative reactor design and optimization of reaction conditions, such as rapid heating and immediate quenching of liquid products.

In the literature, HCW technology is widely reported as a pretreatment method for the fractionation of lignocellulosic biomass. The earlier work pioneered by Bobleter et al.[40−43] and later by Mae et al.[44] demonstrated that HCW pretreatment can effectively remove hemicellulose and lignin from biomass at a temperature as low as 180 °C. Bobleter's group also proposed a two-step HCW pretreatment technology for biomass fractionation,[41] which was later demonstrated by Ando et al.[45] and Saka et al.[46] The two-step technology is effective to completely overcome the recalcitrance of lignocellulosic biomass and recover the sugars from both hemicellulose and cellulose in lignocellulosic biomass. Based on the previous works in the literature[17,40−45,47,48] and this work, this study therefore summarizes a process which combines the two-step HCW pretreatment and subsequent enzymatic hydrolysis technology for rapid production of bioethanol from lignocellulosic biomass. As illustrated in Figure 8, the first step of HCW pretreatment is to recover the sugars from hemicellulose at mild conditions (i.e., 180−230 °C), and the second step of HCW pretreatment

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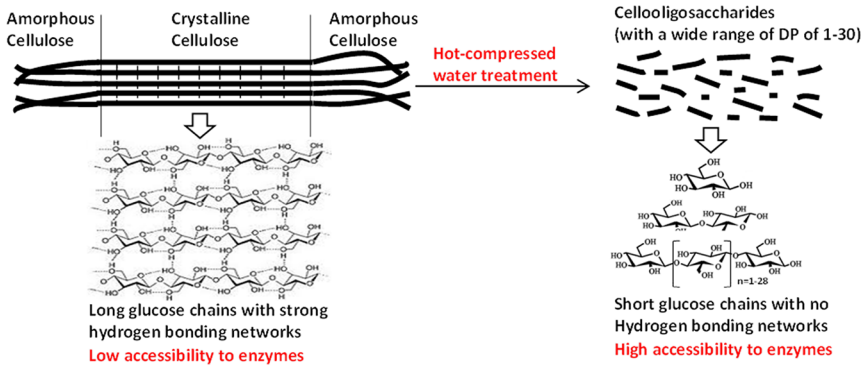

Figure 7. A schematic diagram to break down microcrystalline cellulose into glucose oligomers by HCW pretreatment for rapid recovery of fermentable sugars.

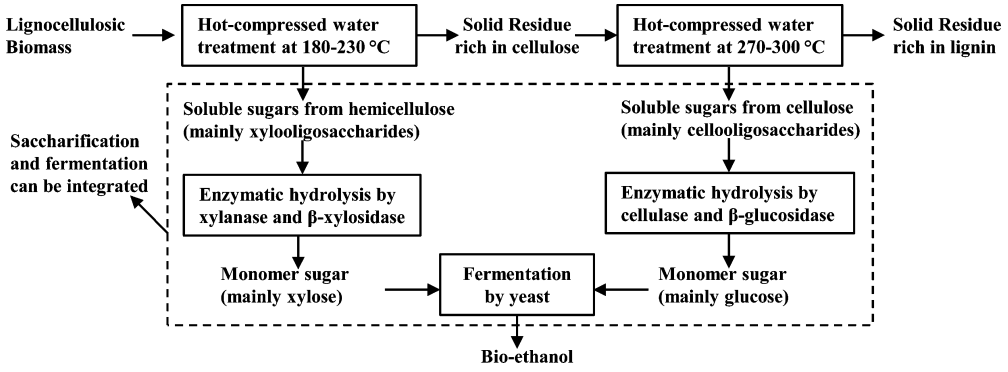

Figure 8. A two-step HCW pretreatment technology to improve the sugar recovery from lignocellulosic biomass for bioethanol production.

is to recover the sugars from cellulose at severe conditions (i.e., 270−300 °C). Then, the recovered sugars from hemicellulose and cellulose can be rapidly hydrolyzed to monomer sugars by suitable enzymes, i.e., xylanase and β-xylosidase for hemicellulose-derived sugars, cellulase and β-glucosidase for cellulose-derived sugars. The enzymatic hydrolysis of hemicellulose-derived sugars has recently been demonstrated,[49] and this work demonstrated that sugar recovery from cellulose can be significantly improved by HCW pretreatment and subsequent enzymatic hydrolysis. It should be noted that the reaction conditions need to be optimized depending on feedstock properties and reaction configurations. For example, the purpose of the first step is to achieve fractionation of biomass so that cellulose dissolution should be minimized. As cellulose hydrolysis in HCW is strongly dependent on the structure of cellulose,[29,36] the temperature (180−230 °C) must be carefully determined for effective fractionation. Also to minimize the secondary reactions of liquid products from cellulose/biomass hydrolysis in HCW, a semicontinuous reactor is a good option, which was also used in the previous studies.[28,45,46] A continuous reactor with a high temperature and an ultrashort residence time may also be an option.

There are several advantages of the two-step HCW pretreatment technology in the proposed process illustrated in Figure 8 in term of significantly reducing the production cost of second-generation biofuel from biomass. First, the two-step pretreatment can be easily implemented using a single reactor operated at two different temperatures sequentially so that it will not significantly increase the capital cost in the pretreatment step. Second, conversion of cellulose into glucose oligomers enables the hydrolysis reaction to occur homogeneously, eliminating the enzyme adsorption issue and the rate decrease problem at increased conversions.[50] The homogeneous reaction is a key reason leading to the drastic increase in the reaction rate of enzymatic hydrolysis. Therefore, the process can potentially reduce the hydrolysis time from days to hours, substantially reducing reactor footprint hence process costs. Third, the process can also significantly reduce the enzyme use during the enzymatic hydrolysis step. This is important because at present the conventional enzymatic hydrolysis technology requires the synergistic actions of three enzymes including endoglucanases, cellobiohydrolases (or exoglucanases), and β-glucosidases. It was also reported that β-glucosidase is also capable of hydrolyzing glucose oligomers with various DPs.[51] If a suitable enzyme can be developed for enzymatic hydrolysis of the liquid product from cellulose HCW pretreatment, the endoglucanases or cellobiohydrolases activities may be not required in the new cellulase enzymes. Additionally, because of the high reactivity of glucose oligomers,[51] the enzyme loading can also be significantly reduced, further contributing to the reduction in biofuel production cost.

4. CONCLUSIONS

This study demonstrates that HCW pretreatment is a promising technology to break down microcrystalline cellulose into glucose oligomers, which can be easily hydrolyzed by cellulase enzymes to fermentable sugars. The formation of soluble sugar oligomers by HCW pretreatment enables the enzymatic hydrolysis to take place homogeneously, thus eliminating several rate-limiting steps in a traditional enzymatic process, such as initial action to release short glucose chains and enzyme adsorption to solid cellulose. Our results show that a liquid sample from HCW pretreatment can be immediately

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converted into glucose oligomers with DPs of 1−5 without incubation even at a low enzyme loading (i.e., ∼8.6 FPU/g glucan-equivalent). At a high enzyme loading (i.e., ∼140 FPU/g glucan-equivalent), all the glucose oligomers can be converted into glucose and cellobiose after 1 h incubation. The sugar recovery after HCW pretreatment can be increased by up to 2 orders of magnitude, depending on enzyme loading and incubation time. A suitable cellulase enzyme with strong cellobiohydrolase and β-glucosidase activities is able to further increase the glucose yield.

AUTHOR INFORMATION

Corresponding Author

*Phone: +61-8-92667592. Fax: +61-8-92662681. E-mail: h.wu@curtin.edu.au.

Notes

The authors declare no competing financial interest.

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