Green Chemistry

Separation of Short-chain Glucan Oligomers from Molten Salt Hydrate and Hydrolysis to Glucose

Journal:	*Green Chemistry*
Manuscript ID	GC-ART-03-2021-000851.R1
Article Type:	Paper
Date Submitted by the Author:	12-Apr-2021
Complete List of Authors:	Liu, Qiyu; South China University of Technology, School of Chemistry and Chemical Engineering Luo, Song; University of Massachusetts, USA, Chemical Engineering Fan, Wei; University of Massachusetts, USA, Chemical Engineering Ouyang, Xinping; South China University of Technology, School of Chemistry and Chemical Engineering Qiu, Xueqing; South China University of Technology

ARTICLE

Separation of Short-chain Glucan Oligomers from Molten Salt Hydrate and Hydrolysis to Glucose

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Qiyu Liu,[a] Song Luo,[b] Wei Fan,*[b,c] Xinping Ouyang,*[a] and Xueqing Qiu,*[a,d]

Selective production of glucose from hydrolysis of cellulose is the key step for efficient utilization of lignocellulose biomass. Crystalline cellulose can be dissolved and hydrolyzed into glucose with a high selectivity in molten salt hydrates (MSHs). However, the separation of the formed glucose is challenging due to its high solubility in the MSHs. To address this issue, a stepwise method is introduced, where cellulose is hydrolyzed into short-chain glucan oligomers in the MSH of LiBr. We demonstarte that compared to glucose, the formed glucan oligomers with a degree of polymerization of 4-11 can be efficiently separated from the MSH hydrolysate using an anti-solvent precipitation method. The separated oligomers can be readily converted into glucose under mild conditions and used for other applications. Under optimized conditions, 90.3% of glucan oligomer can be produced from crystalline cellulose and separated from the MSH with the addition of methanol, and the precipitated glucan oligomer can be hydrolyzed into glucose with a yield of 99.7% using dilute sulfuric acid. We show that the precipitation efficiency is influenced by the glucan oligomer chain-length, glycoside bond type and concentration. Moreover, separation of glucan oligomer from cotton straw hydrolysate was also investigated in the MSH. 79.2% yield of glucan oligomer was obtained from hydrolysis of cotton straw at 130 °C for 2 h. With the addition of methanol, glucan oligomer was precipitated with the selectivity of 60.8%.

Introduction

Cellulose is the most abundant homopolysaccharide in nature which consists of D-glucose units connected through β(1→4) glycosidic bonds.[1] Selective production of glucose from hydrolysis of cellulose is the key step for efficient conversion of lignocellulosic biomass into fuels and value-added chemicals.[1, 2] Crystalline cellulose obtained from lignocellulosic biomass has a high degree of polymerization (DP), together with massive inter- and intramolecular hydrogen bonds in the structure, resulting in low solubility in water and limited accessibility to homogeneous and heterogeneous catalysts as well as cellulolytic enzymes.[3, 4] In order to address this issue, several approaches have been developed.[3, 5] One promising approach is to apply specific solvents, including ionic liquids,[6, 7] deep eutectic solvents[8] and molten salts hydrates (MSHs),[9, 10] to dissolve and partially depolymerize crystalline cellulose. These solvents break the hydrogen bonds in cellulose structure,[10-12] therefore, increasing cellulose solubility in the solvents and possibly facilitates catalytic hydrolysis of cellulose. Selective production of glucose from cellulose has been achieved by catalytic hydrolysis using acid catalysts in the solvents.[7, 13-15]

Despite the promising potential for selective production of glucose from cellulose using ionic liquid, deep eutectic solvent and MSHs, there is a significant challenge in separation of the produced sugars.[16, 17] **Table 1** summarizes representative studies of cellulose hydrolysis in ionic liquids, deep eutectic solvents and MSHs. It was found that cellulose can be effectively swollen, dissolved and partially depolymerized in those solvents at mild conditions, but separation remains the major challenge[18, 19] (row 1 to 4). Physical adsorption of produced sugar on amorphous carbon and zeolites have been reported (row 5 and 6), however the desorption of the sugars from the adsorbents requires additional steps.[20] Membrane separations have also been used for concentrating sugars from hydrolysates of cellulose (row 7). Compared to cyclic temperature swing adsorption processes using solid adsorbents, membrane separations including pressure driven nanofiltration (NF) and forward osmosis (FO) separations are easily scalable in continuous processes.[21, 22] Membrane separations are often used to obtain concentrated sugars stream from lignocellulosic biomass pre-treatment in which salts are not used. In the presence of high concentration of salts, the selectivity for sugar over salts through the membrane separations need to be further demonstrated. In addition, fouling effects are also needed to be investigated. Ion exchange method is another promising method in sugar separation from ionic liquid (row 8) but the regeneration of resin is challenging.[23]

[a] School of Chemistry and Chemical Engineering, South China University of Technology, Guangzhou, 510640, China.
E-mail: cexqqiu@scut.edu.cn.
[b] Department of Chemical Engineering, University of Massachusetts-Amherst, 159 Goessman Lab, 686 N Pleasant Street, Amherst, 01003, USA.
E-mail: wfan@engin.umass.edu
[c] Catalysis Center for Energy Innovation, a U.S. Department of Energy-Energy Frontier Research Center, University of Delaware, Newark, Delaware 19716, USA.
[d] School of Chemical Engineering and Light Industry, Guangdong University of Technology, Guangzhou, Guangdong 510006, China.
†Electronic Supplementary Information (ESI) available.

ARTICLE

Table 1 Cellulose conversion in ionic liquids, deep eutectic solvents and MSHs

Solvents	Substrate	Catalysts	Temp.	Time	Products	Yields	Separation	References
$[C_4min]^+$ salts [a]	Cellulose	None	70 °C	30 min	TRS [b] and glucose	62% and 14%	No	Ref.1[24]
[HMIM][HSO_4]	Cellulose	$MnCl_2$	150 °C	300 min	5-HMF	41.7%	No	Ref.2[25]
L-Proline/Ethylene glycol	Cellulose	$FeCl_3$•$6H_2O$	120 °C	120 min	TRS	66.7%	No	Ref.3[26]
LiBr•3.25 H_2O	Cellulose	H_2SO_4	85 °C	30 min	Glucose	82%	No	Ref.4[14]
LiBr•3.25 H_2O	Cellulose	None	130 °C	5 h	Glucan oligomer	90.4%	Yes [c]	Ref.5[20]
70 wt.% $ZnCl_2$	Cellulose	HCl	70 °C	60 min	Glucose	~84%	Yes [d]	Ref.6[27]
50 wt.% LiCl	Sugar cane bagasse	HCl	80 °C	60 min	Glucose	79.1%	Yes [e]	Ref.7[28]
$[C_4mim]Cl$	Cellulose	H_2SO_4	100 °C	9 min	TRS	68%	Yes [f]	Ref.8[23]
LiBr•3.25 H_2O /Ethyl acetate	Poplar wood	H_2SO_4 and $AlCl_3$	120 °C	1 h	Furfural and 5-HMF	>80 mol%	Yes [g]	Ref.9[29]
LiCl•$3H_2O$/methyl isobutyl ketone	Cellulose	$NbOPO_4$/HZSM-5	175 °C	2 h	Levulinic acid	94.0%	Yes [h]	Ref.10[30]
BMIMCl	Cellulose	Amberlyst-15 and p-TSA	100 °C	5 h	Glucan oligomers	48%	Yes [i]	Ref.11[7]
DES [j]	Cellulose	None	100 °C	2 h	Cellulose nanocrystals	66-71%	Yes [k]	Ref.12[8]
$ZnBr_2$•4 H_2O	Cellulose	None	70 °C	15 min	Amorphous cellulose	99%	Yes [l]	Ref.13[31]

[a] Anions are Cl^-, Br^- and SCN^-. [b] TRS refers to total reducing sugar. [c] Separation was performed by adsorption on amorphous carbon. [d] Separation was performed by adsorption on zeolite beta. [e] Separation was performed using nanofiltration. [f] Separation was performed by ion exchange. [g] Separation was performed by extracting furfural and 5-HMF using ethyl acetate. [h] Separation was performed by extracting levulinic acid using methyl isobutyl ketone. [i] Separation was performed by adding water as anti-solvent. [j] DES was prepared by mixing choline chloride and oxalic acid dihydrate. [k] Separation was performed by adding water as anti-solvent. [l] Separation was performed by adding water as anti-solvent.

Extraction of glucose from MSH using boronic acid has shown promising results, however, the efficiency and cost of the method requires further evaluation. Another possible approach is to upgrade glucose to other valuable chemicals in the solvents followed by biphasic separation or other separation methods (row 9 and 10).[29, 30] Despite these efforts, maintaining the

glucose structure is still highly desired because of their value and versatile conversion pathways sugars provide. Precipitation using anti-solvents is simple, easily scalable and consumes low energy compared to adsorption-based separation and membrane separation.[31] However, due to the high solubility of monosaccharide in ionic liquids, deep eutectic solvents and MSHs, the precipitation method cannot be used for separating glucose. Attempts have been made to separate oligomers with high DP of more than 300 or partially crystalline cellulose (row 11 to 13). Production of glucose from the partially crystalline cellulose still requires relatively harsh reaction conditions and presents relatively low yields. Moreover, due to the complex structure of cellulose, the effects of anti-solvent on the precipitation efficiency have not been fully understood.

Glucan oligomer is a carbohydrate polymer with a low DP (commonly 2-10).[32, 33] Crystalline cellulose can be selectively depolymerized into glucan oligomers with a yield of 90.4% in MSHs.[20] The glucan oligomers are solvated and soluble in MSHs, and can be readily converted into glucose under mild conditions with a high selectivity.[20, 34] It has been known that the solubility of glucan oligomers in water rapidly decreases with the increase of glucan chain length.[35-37] For example, at room temperature glucose and cellobiose show solubilities of 47.8 wt.% and 6.8 wt.% in water, respectively, while the solubility of linear β-glucan oligomers with DP = 5-6 is less than 1 wt.% in water.[35, 38] Motivated by the low solubility of glucan oligomer in water compared to glucose, we argue that the solubility of glucan oligomers in MSHs also decreases with their chain length. Compared to glucose, precipitation of glucan oligomers with the DP of 4 to 11 from MSHs using anti-solvents should be feasible. Therefore, a stepwise hydrolysis process is examined in this study, where cellulose is selectively hydrolyzed into glucan oligomers in MSHs, and the oligomers are separated via anti-solvents precipitation followed by hydrolysis into glucose under mild conditions. Our choice of separating glucan oligomers rather than glucose from MSH stems from not only being solvated and dissolved in MSH but importantly they can be precipitated from MSH at a much lower concentration than glucose, offering an improved separation efficiency. Compared with the previous work, the anti-solvent precipitation method can reach the limitation of unable separation of glucose[19] and insufficient separation of branched glucooligosaccharides[32] from MSHs. Additionally, oligomer is also considered as a value-added product with unique applications in polymer production, agriculture, and food science.[39-41] This study proposes a simple and economic process for separation of glucan oligomers and production of glucose from cellulose which is regarded as a bottleneck for the application of lignocellulosic biomass. Hydrolysis of cotton straw and separation of produced glucan oligomers were also studied in this work, providing a potential for direct utilization of the method for raw biomass.

Experimental section

Materials

Glucose (99.9%), microcrystalline cellulose (99%, 90 um), methanol (MeOH, 99.9%), γ-valerolactone (GVL, 98+%), dimethyl formamide (DMF, 99%), pyridine (anhydrous, 99.5+%) and phenyl isocyanate (98+%) were purchased from Alfa Aesar, Ltd., USA. Lithium bromide (LiBr, 99%) and D_2O (99.9%) were purchased from Sigma-Aldrich, Ltd., USA. Ethanol (EtOH, 99.5%), isopropanol (IPA, 99.9%) and tetahydrofuran (THF, 99.9%) were purchased from Fisher Scientific. Cotton straw was produced from Jolgaon, India. The raw biomass was directly used after being grinded and sieved through a 60-mesh sieve.

Glucan oligomer preparation

Molten salt hydrate was prepared by dissolving 3.6 g of LiBr in 2.4 g of deionized water, forming a MSH with 60 wt.% of LiBr. Hydrolysis reaction was carried out by mixing 100 mg of microcrystalline cellulose with 6 g of MSH in a 15 mL thick wall glass reactor (Synthware Ltd., China). The hydrolysis reaction was performed at 130 °C for 5 h in a preheated oil bath with a magnetic stirring at 300 rpm. A glucan oligomer yield of 90.4% was obtained.[20] The hydrolysate was collected for the following precipitation study.

Measurement of glucose and glucan oligomer concentrations

Concentration of glucose in the MSH was detected by high performance liquid chromatography (HPLC) after 10 times dilution of the solutions. The HPLC (LC-20AT, Shimadzu) was equipped with refractive index (RID-10A) detector at an oven temperature of 85 °C. A Bio-Rad HPX-87H HPLC column with a guard column was used. HPLC grade dilute sulfuric acid (0.1 mmol/L, Fisher) was used as a mobile phase with a flow rate of 0.6 mL/min.

The method for measuring the concentration of glucan oligomers in the MSH has been introduced in our previous work.[20] Namely, 1 g of hydrolysate was mixed with 5 g of 4 wt.% H_2SO_4 in a 15 mL thick wall glass reactor and hydrolyzed at 130 °C for 1 h with stirring. The formed glucose solution was filtered by 0.22 um PTFE syringe filter and measured by HPLC.

Measurement of the solubility of sugars in different anti-solvents

The solubility of glucose, maltose, cellobiose in MeOH and glucan oligomer in different anti-solvents were investigated, respectively. The glucan oligomers were obtained using our previous method.[20] During the solubility measurements, 20 mg of sugar was mixed with 1 g of solvent in a 3 mL glass vial. The vials were heated on a hot plate with magnetic stirring at 600 rpm at 30 °C for 48 h. Thereafter, the solution was filtered by 0.22 um PTFE syringe filter to separate the insoluble part. The amount of glucose, maltose and cellobiose dissolved in the solutions were detected by HPLC. The amount of glucan oligomers dissolved in the solution were measured by HPLC after hydrolyzing the dissolved glucan oligomers by 4 wt.% H_2SO_4 in a 15 mL thick wall glass reactor at 130 °C for 1 h. The solubility of sugar was calculated using eqn.1.

$$\text{Sugar solubility } (S_S) = \frac{W_{DS}}{W_S} \times 100\% \quad (1)$$

here W_{DS} is the weight of dissolved sugar in the solvent. W_S is the weight of the solvent.

Hydrolysis of cotton straw in MSH

The composition of cotton straw was determined by the method reported in our previous study [42, 43] which was established by National Renewable Energy Laboratory (NREL).[44] Essentially, 0.3 g of dried biomass was mixed with 3 mL of 72% H_2SO_4 in a 25 mL beaker and placed in a water bath at 30 °C for 1 h. Then 84 mL of deionized water was added into the mixture to dilute the acid concentration to 4% and reacted at 121 °C for 1 h. The hydrolysate was detected by HPLC. Cellulose and hemicellulose amount were calculated based on soluble monosaccharides amount. The insoluble part after hydrolysis consists of lignin and ash. The amount of ash was determined by placing the filter in a furnace at 575 °C for 4 h. The quality loss during the process was lignin. The composition of cotton stover was measured as: 34.7% of cellulose, 20.4% of hemicellulose, 19.9% of lignin, and 25.0% of others.

For cotton straw hydrolysis in the MSH, 300 mg of cotton straw was mixed with 6 g of the MSH in a 15 mL thick wall glass reactor with magnetic stirring at 600 rpm. The reactor was put into an oil bath for hydrolysis. After reactions, the reactor was taken out from the oil bath and rapidly cooled down to room temperature with cooling water. After the hydrolysis reaction, the hydrolysate was separated from solid residue by 0.22 um PTFE syringe filter. The glucose and glucan oligomer concentrations in the hydrolysate were measured by HPLC. Unreacted cellulose in the solid residue was dried in the oven at 80 °C for 12 h before being measured by the method developed by NREL which was mentioned above. Glucose concentration in the solution was measured by HPLC.

The yields of different products in the hydrolysates obtained from cellulose hydrolysis are calculated using eqns. (2-4):

$$\text{Glucose yield } (Y_{GB}) = \frac{M_{GH}}{M_{GB}} \times 100\% \quad (2)$$

$$\text{Oligomer yield } (Y_{OB}) = \frac{M_{GHO}}{M_{GB}} \times 100\% \quad (3)$$

$$\text{Unreacted cellulose yield } (Y_{UCB}) = \frac{M_{GHR}}{M_{GB}} \times 100\% \quad (4)$$

Here M_{GH} is the mole of glucose in the hydrolysate after the hydrolysis of cotton straw in the MSH. M_{GHO} is the mole of glucose units in the oligomers formed in the MSH hydrolysis of cotton straw. M_{GHR} is the mole of glucose units in the residual cellulose. M_{GB} is the total mole of glucose units in the cotton straw. The products obtained from hemicellulose hydrolysis including xylan oligomer, xylose and unreacted hemicellulose were analyzed in the same way as cellulose. All data points were repeated for three times and error bars were made by dividing the standard deviation by the square root of 3.

Precipitation of products using anti-solvents

Anti-solvent precipitation was used to separate the soluble glucan oligomers formed from hydrolysis of cellulose and cotton straw in the MSHs. After hydrolysis in the MSHs, the hydrolysates were obtained after removing the undissolved solids by filtration. In the anti-solvent precipitation process, 1 g of the hydrolysate was added into varying amounts of anti-solvents. After stirring for 5 h at room temperature, white solids were precipitated out from the MSHs. To fully separate the solids, the solution was centrifuged at 6,000 rpm (3823 RCF*g) for 10 min with a centrifuge (Xiangyi H1850, China). The supernatant was removed, and the precipitated glucan oligomers were isolated.

The amount of residual glucan oligomers in the MSH and the precipitated glucan oligomers were determined by HPLC after hydrolysis into glucose.

The precipitation yield of glucan oligomer from microcrystalline cellulose hydrolysis is calculated using eqn.5:

$$\text{Precipitation yield } (Y_{POC}) = \frac{M_{GOC} - M_{GPOC}}{M_{GOC}} \times 100\% \quad (5)$$

here M_{GPOC} is the moles of glucose units in residual glucan oligomers dissolved in the precipitation solution. M_{GOC} is the moles of glucose units in total oligomers in the hydrolysates after hydrolysis of cellulose in the MSH.

The precipitation yield of oligomer from biomass hydrolysis is calculated using eqn.6:

$$\text{Precipitation yield } (Y_{POB}) = \frac{M_{GOB} - M_{GPOB}}{M_{GOB}} \times 100\% \quad (6)$$

here M_{GPOB} is the moles of glucose units in residual glucan oligomers dissolved in the precipitation solution. M_{GOB} is the moles of glucose units in the total oligomers in the hydrolysates after hydrolysis of cotton straw in the MSH. All data points were repeated for three times and error bars were made by dividing the standard deviation by the square root of 3.

Solubility of sugars in MSH and precipitation performance

Different amounts of glucose, maltose and cellobiose were added into MSH followed by stirring at room temperature for 1 h. The solution was filtered using 0.22 um PTFE syringe filter to remove the undissolved components. The sugar concentration in the solutions was measured by HPLC. To evaluate precipitation efficiency using the anti-solvent, MeOH, 1 g of the hydrolysate after removing the undissolved component was added into 10 g of MeOH. After stirring for 5 h at room temperature, the solution was centrifuged at 6,000 rpm (3823 RCF*g) for 10 min. Then the supernatant was filtered by 0.22 um PTFE syringe filter to measure the amount of dissolved sugar by HPLC.

The sugar concentration in MSH is calculated using eqn.7:

$$\text{Sugar concentration } (C_S) = \frac{W_S}{W_T} \times 100\% \quad (7)$$

Here W_S is the weight of sugar dissolved in MSH, W_T is the total weight of the solution.

The sugar precipitation yield after the addition of MeOH is calculated using eqn.8:

$$\text{Precipitation sugar yield } (Y_{PS}) = \frac{W_{DS} - W_{RS}}{W_{DS}} \times 100\% \quad (8)$$

Here W_{DS} is the weight of dissolved sugar in MSH, W_{RS} is the weight of residual sugar dissolved in MSH after the addition of MeOH.

^{1}H-NMR analysis of glucan oligomers from cellulose hydrolysis

To understand the structure of glucan oligomers obtained from cellulose hydrolysis in the MSH. ^{1}H-NMR spectra were collected for the detection of the prevalence of glycosidic linkages and reducing ends. The sample was prepared by dispersing 5.0 wt.% dried oligomers in D_2O with 10 mM 4,4-dimethyl-4-silapentance-1-sulfonic acid (DSS) as an internal standard. ^{1}H-NMR samples were sonicated for 15 min and vortexed for 3 min followed by filtration before the measurement. The ^{1}H-NMR measurement was performed with 32 scans. Peaks from 5.6 to 4.0 ppm δ range was analyzed in this work. The relative peak positions of the hydrogens on α and β reducing ends (α-RE and β-RE), α(1→4), α(1→6), β(1→4) anomeric hydrogens which have been reported in previous literatures.[35, 45] Abundance was calculated by a comparison of the integrated individual peak areas (A_{Hi}) in the ^{1}H-NMR spectra:

$$Abundance = \frac{A_{Hi}}{\Sigma A_{Hi}} \times 100\% \quad (9)$$

Results and discussion

Glucan oligomer structural characterization

The glucan oligomers obtained from cellulose hydrolysis in the MSH of LiBr consist of a series of short-chain glucan oligomers with DP ranging from 4 to 11 (**Figure 1a**).[20] ^{1}H-NMR was employed to identify the internal anomeric hydrogen of the oligomers. The ^{1}H-NMR spectrum is shown in **Figure 1b** and the anomer distribution is listed in **Table 2**. The result indicates that 93.2% of anomeric hydrogens in the oligomers are associated with β(1→4) linkages, and no α(1→4) and α(1→6) linkages were detected. It has been known that the solubility of glucan oligomers consisting of β(1→4) linkage is much lower than the oligomers formed by α(1→4) and α(1→6) linkage in aqueous phase,[36] making it possible to precipitate glucan oligomers with β(1→4) linkage by anti-solvent precipitation.

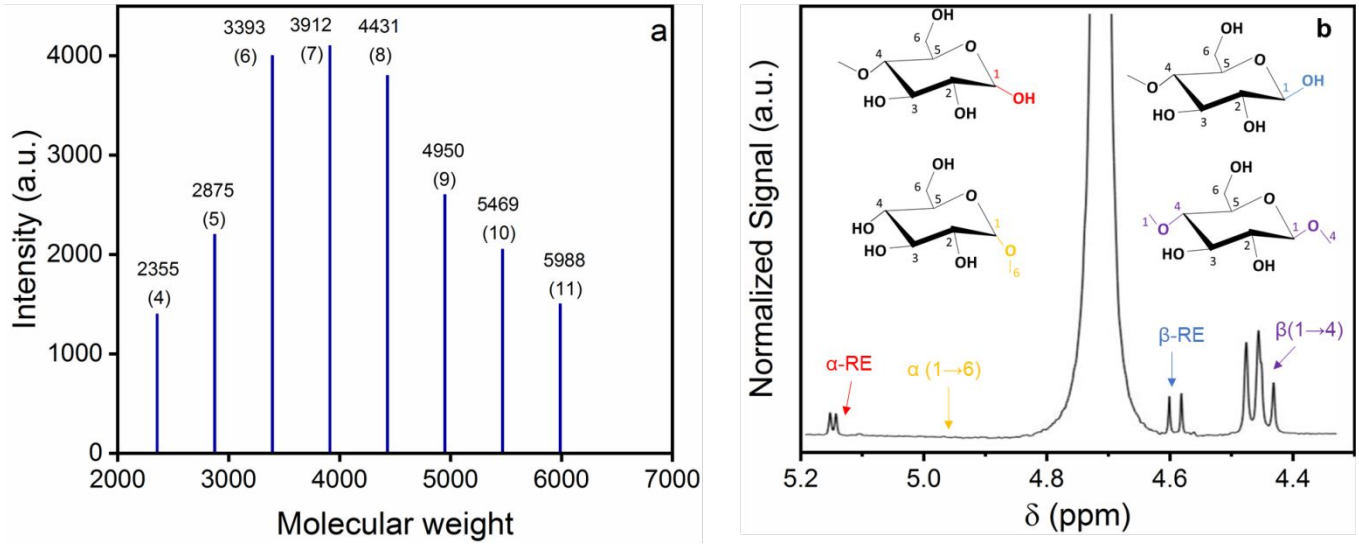


Figure 1. (a) Molecular weight of glucan oligomers (outside the brackets) and number of glucose unit (DP, inside the brackets)[20] and (b) ^{1}H-NMR spectra of glucan oligomer obtained from cellulose hydrolysis in MSH at 130 °C for 5 h.

Table 2 Anomeric glucan oligomer abundance calculated from ^{1}H-NMR [a]

Anomer	α(1→4)	α-RE	α(1→6)	β-RE	β(1→4)
Percentage	0.0	1.3	0.0	5.5	93.2

[a] Glucan oligomers preparation: Crystalline cellulose hydrolysis in MSH at 130 °C for 5 h.

Effect of different solvents on glucan oligomer precipitation

After formation of the short-chain glucan oligomers by cellulose hydrolysis in the MSH, anti-solvent precipitation was employed to separate the oligomers. Anti-solvents evaluated in the study are miscible with the MSH and exhibit a low solubility for the glucan oligomers. The precipitation yield and oligomer solubility in different anti-solvents including MeOH, EtOH, IPA, THF, GVL, and DMF are shown in **Figure 2**. The result shows that 90.3% yield of oligomer was precipitated out from the MSH using MeOH and the other 9.7% of oligomer dissolved in the mixture of MSH and MeOH. When EtOH and IPA were employed in the

precipitation process, 79.6% and 78.5% yields of precipitated oligomers were obtained. Negligible oligomer solubilities in those three alcohols are observed as shown in **Figure 2,** but the precipitation yield in MeOH is slightly higher than EtOH and IPA. The reason may be due to the fact that MeOH has a higher polarity (0.762) than EtOH (0.654) and IPA (0.546), which means MeOH can form stronger interaction with the MSH and weaken the interaction between the oligomers and the MSH, facilitating oligomer precipitation.[15] Besides, a series of solvents commonly emloyed in biomass conversion including THF, GVL and DMF were also studied as anti-solvents for the precipitation study, respectively. It was found that precipitation yields of 76.1% and 50.9% were achieved with the addition of THF and GVL, and the oligomer solubilities in those two solvents are 0.06 wt.% and 0.09 wt.%, respectively. These results indicate that effective anti-solvents should have a low solubility for the glucan oligomers. Moreover, the addition of DMF doesn't show any oligomer precipitation. The reason is due to the fact that DMF can form specific interactions with LiBr solution and increases oligomer solubility, which is similar to the cellulose dissolution in the solution of DMF/LiCl/H_2O.[46] Among the six anti-solvents studied above, MeOH presented the highest precipitation efficiency. The higher precipitation efficiency achieved from MeOH is likley caused by the low solubility of oligomers in MeOH and strong interaction between MeOH and the MSH. Additionally, the effect of methanol dosage on glucan oligomer precipitation efficiency was also investigated. The result indicates that the precipitation yield depends on the MeOH/MSH weight ratio. When the MeOH/MSH weight ratio changes from 2 to 10, the precipitation yield was increased from 68.3% to 90.3% (**Figure S1**). Further increase of the MeOH dosage cannot enhance the precipitation efficiency. Thus, in this work, 10 times of MeOH was added into MSH for the purpose of achieving a sufficient precipitation result.

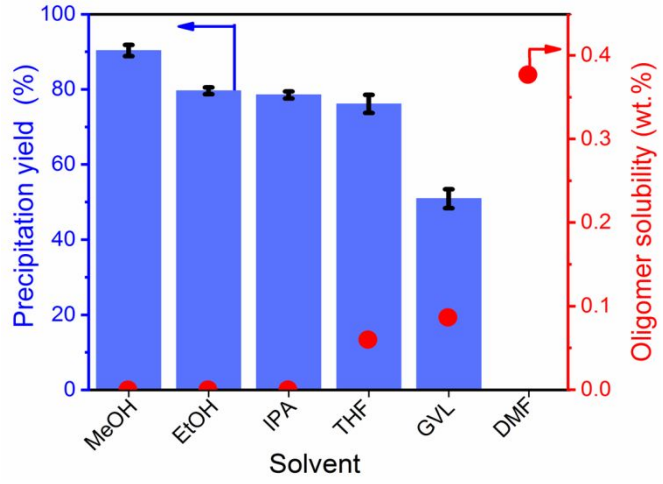


Figure 2. Precipitation yield of glucan oligomer with the addition of different anti-solvents and the solubility of oligomers in different anti-solvents. Precipitation condition: 1 g of hydrolysate added in 10 g of anti-solvents. Dissolution condition: 20 mg of solid oligomer was added into 1 g of solvent, stirring at 30 °C for 48 h.

Sugar dissolution in the MSH and precipitation efficiency

In order to further understand the effect of anti-solvent on the precipitation of sugars from the MSH, saccharides with different glucosidic linkages and chain lengths were investigated, including glucose (monosaccharide), cellobiose (disaccharide with β(1→4) glucosidic linkage), maltose (disaccharide with α(1→4) glucosidic linkage) and glucan oligomers (products from cellulose with DP of 4-11 linked by β(1→4) glucosidic linkages).

The precipitation efficienty of different saccharides from the MSH using anti-solvent, MeOH, is summarized in **Figure 3** and the pictures of the samples after precipitation are shown in **Figure S2** with detailed data listed in **Table S1**. As shown in **Figure 3**, the linear glucan oligomer with β(1→4) linkage can precipitate from the MSH hydrolysate with MeOH addition at a concentration as low as 1.64 wt.%. On the contrary, glucose can not be precipitated with MeOH addition in the studied concentration range, which is due to the high solubility of glucose in the MSH.[19] Disaccharides including cellobiose and maltose also show negligible precipitation when the saccharide concentrations are lower than 5 wt.%. For 5 wt.% of cellobiose in the MSH, 17.3 wt.% of cellobiose can be precipitated. Maltose is more difficult to be precipitated. No precipitation was observed at 5 wt.% of maltose. When the maltose concentration changed from 10 wt.% to 40 wt.%, the precipitation yield of maltose was gradually increased from 10.4 wt.% to 40.2 wt.%. The difference in the precipitation yield of glucose, cellobiose and oligomer suggests that the precipitation efficiency increases with increasing the chain-length of the saccharides. Therefore, the anti-solvent precipitaton method can be used for separation of glucan oligomers at a concentration of as low as 1.64 wt.% with a yield above 90%. The precipitation results of cellobiose and maltose indicate that the saccharide with β(1→4) linkage is easier to separate compared to the saccharide with α(1→4) linkage. This suggests that the separation of linear glucan oligomers with β(1→4) linkage prepared by cellulose hydrolysis in the MSH is more efficient than separating branched oligomers with α(1→4) linkage.[32]

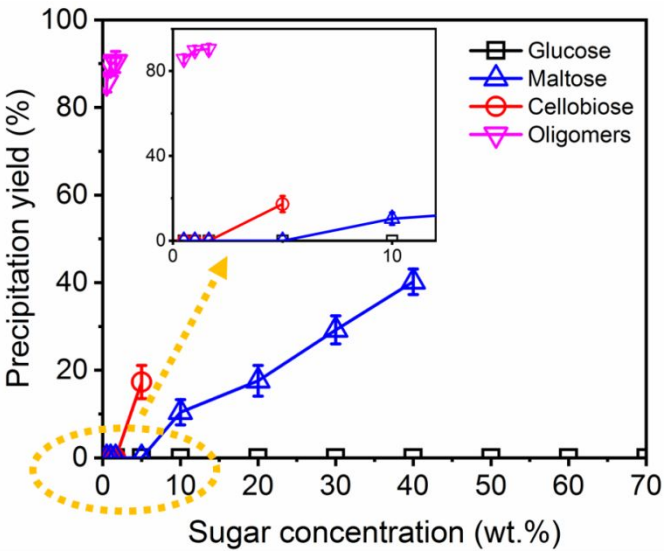


Figure 3. Precipitation yields of sugars from the MSH of LiBr using MeOH as the anti-solvent. Precipitation conditions: 1 g of sugar solution mixed with 10 g of MeOH.

The solubility of different saccharides in the MSH of LiBr and MeOH were studied in order to investigate the precipitation efficiency. The solubility of the saccharides in the MSH was measured by adding different amounts of the saccharides into the MSH after removing undissolved part by filtration. The status of the solutions is shown in **Figure S3**. The concentration of the saccharides in the solutions measured by HPLC and amount of the saccharides initially added into the MSH was plotted in the parity plot of **Figure 4a**. It indicates that glucose can be readily dissolved in the MSH with a concentration as high as 70 wt.% (**Figure S3a**). When maltose was employed in the dissolution study, the concentration of maltose in the MSH can reach to around 40 wt.%. When 50 wt.% of maltose was added into the MSH, the maltose can be well dispersed, but the solution exhibits a high viscosity and cannot go through the 0.22 um PTFE syringe filter (**Figure S3b**). Cellobiose can be dissolved in the MSH when the concentration is lower than 5 wt.%. The undissolved solid was observed when the cellobiose concentration further increases (**Figure S3c**). The results support the claim that the solubility of saccharides in the MSH decreases with the chain length, and saccharides with α(1→4) linkage is prone to dissolve in the MSH compared to the saccharides with β(1→4) linkage. The glucan oligomer can be dissolved in the MSHs up to 1.64 wt.%, which is corresponding to the glucan oligomer concentration in the hydrolysate prepared by cellulose hydrolysis in the MSH. The result suggests that the glucan oligomers produced from the cellulose hydrolysis in the MSH can be fully dissolved in the MSH. Further increase in glucan oligomer concentration in the hydrolysates is possible by increasing the amount of cellulose added into the MSH. However, our previous study showed that the selectivity to glucan oligomer from hydrolysis of cellulose decreased with increasing the amount of cellulose in the MSH.[20] Optimized reaction conditions for the hydrolysis led to a yield 1.64 wt.% of glucan oligomer with a selectivity of 90.4%.

The solubility of different saccharides in MeOH was analyzed and listed in **Figure 4b**. All saccharides show low solubilities in MeOH. Glucose and maltose present a similar solubilities of 0.23 and 0.19 wt.%, while cellobiose and oligomer present negligible solubilities, respectively. The solubilities of glucose, cellobiose and oligomer in MeOH indicate that the increase of chain-length has negative effects on their solubilities in MeOH, which is the same as in water.[35] The solubility difference between maltose and cellobiose suggests the disaccharide with α(1→4) linkage is easier to dissolve in MeOH compared with disaccharide wth β(1→4) linkage.

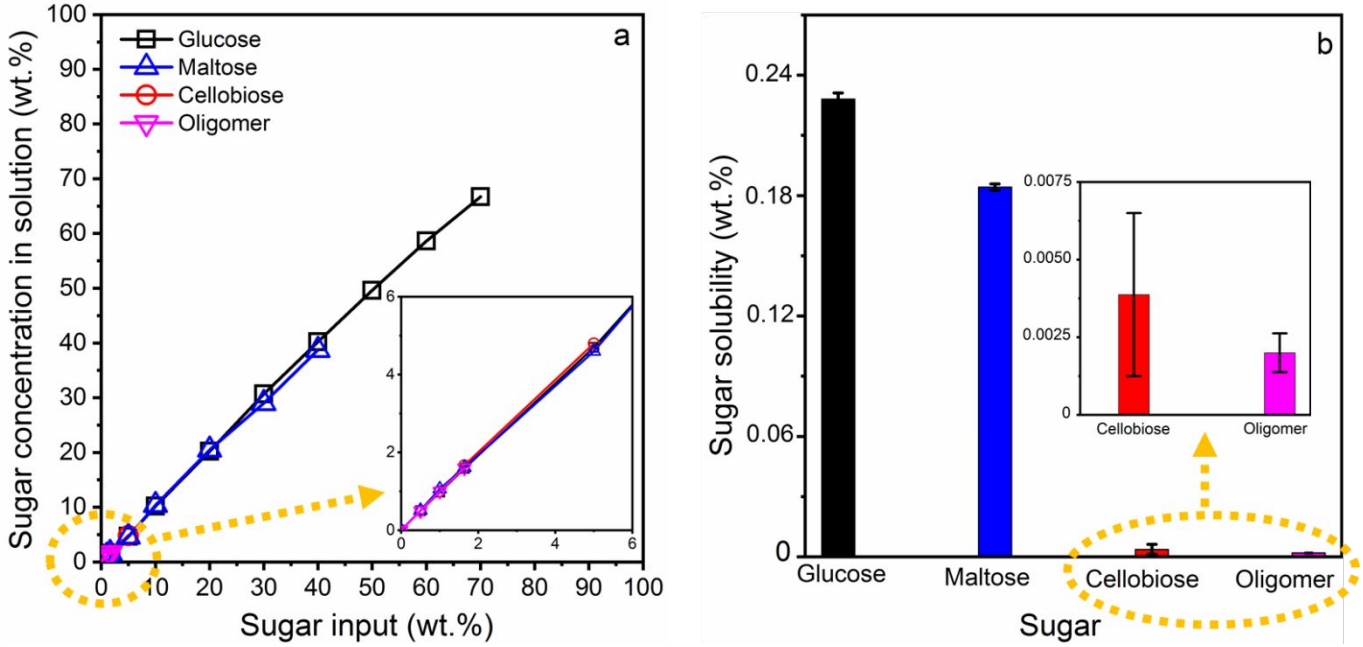


Figure 4. (a) Dissolution of saccharides with different concentrations in the MSH of LiBr, and (b) the solubility of different saccharides in MeOH.

Hydrolysis of precipitated glucan oligomers

The precipitated glucan oligomers were hydrolyzed into glucose using dilute sulfuric acid at 130 °C. Microcrystalline cellulose was hydrolyzed under the same conditions as controlled experiments. According to the hydrolysis results shown in **Figure 5**, the crystalline cellulose presented glucose yields from 0.3% to 3.7% with the reaction time increasing from 10 min to 60 min, suggesting an insufficient hydrolysis at 130 °C. When the precipitated oligomer was hydrolyzed under the reaction conditions, glucose yields increase from 24.4% to 99.7% when reaction time increases from 10 min to 40 min. The results indicate that the precipitated oligomer can be selectively hydrolyzed into glucose compared with crystalline cellulose at the mild reaction temperature of 130 °C, which is due to its short-chain structure and easy access to the acid catalyst. The easy separation of the glucan oligomers from the MSH, the high selectivity to glucose and mild reaction conditions for hydrolysis of the glucan oligomers provide a promising way to selectively convert crystalline cellulose to glucose.

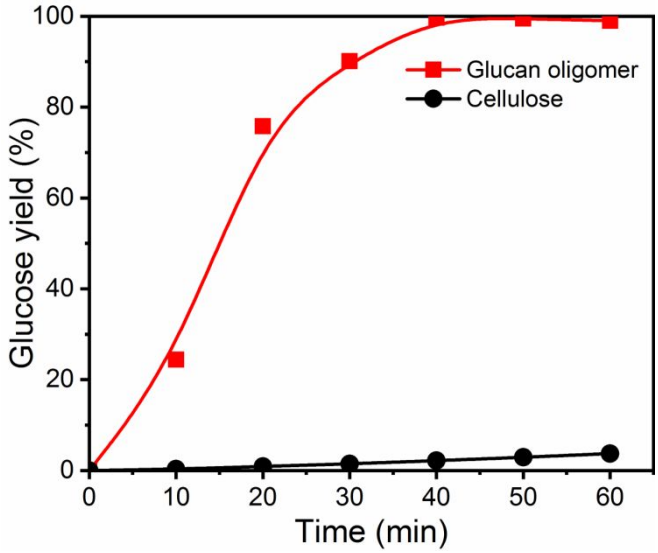


Figure 5. The glucose yield produced from the hydrolysis of precipitated glucan oligomers and microcrystalline cellulose by dilute sulfuric acid. Hydrolysis conditions: 40 mg of substrate, 5 g of 4 wt.% H_2SO_4, 130 °C.

Hydrolysis and precipitation of glucan oligomers from cotton straw in MSH

Cotton straw was investigated as raw biomass for producing glucan oligomers from hydrolysis in the MSH and anti-solvent precipitation. In order to optimize the glucan oligomer yield from the hydrolysis of cotton straw, the reactions were carried out at two different temperatures. The result in **Figure 6a** shows that the cellulose hydrolysis continuously proceeds at 120 °C with time increased from 1 h to 4 h. During the process, cellulose content decreased from 92.2% to 39.0% with glucan oligomers increased from 7.5% to 60.5%, indicating a selective conversion from cellulose to glucan oligomers. When the reaction was carried out at 130 °C, a faster depolymerization was observed. Cellulose was hydrolyzed into glucan oligomer with a yield of 77.5% at 2 h and then totally hydrolyzed at 3 h with a glucan oligomer yield of 85.2%. Further increase of reaction time to 4 h led to a decreased glucan oligomer yield to 69.2% and a glucose yield of 20.4%.

Due to the relative high yield of glucan oligomers achieved from the hydrolysis at 130 °C, the hydrolysates obtained at 1 h, 2 h, 3 h and 4 h were employed for anti-solvent precipitation. As shown in **Figure 6b**, with the addition of MeOH, 79.2% of glucan oligomers were precipitated from the hydrolysate obtained after 1 h of hydrolysis. With the increase of hydrolysis time, the precipitated yields of glucan oligomers gradually decreased. For the hydrolysates obtained at 2h, 3 h and 4 h, only 60.8%, 47.3% and 26.2% of glucan oligomers were precipitated, respectively. The decreased precipitation yield with increasing the hydrolysis time is due to the fact that the further cleavage of the β(1→4) glycosidic bonds of the glucan oligomers, resulting in a decrease in the chain-length of glucan oligomer. The produced oligomers with a shorter chain-length have a higher solubility in the mixture of MSH and MeOH than the oligomers formed in the early stage of the hydrolysis which have a longer chain-length. It is more difficult to precipitate the oligomers with a relatively short chain-length using the anti-solvent precipitation method. Hydrolysis and precipitation of hemicellulose in the cotton straw was also investigated. The highest xylan oligomer yield of 78.2% was obtained after hydrolysis in the MSH at 130 °C for 2 h **(Figure 6c)**, and the precipitation yield of 53.2% was obtained with MeOH addition **(Figure 6d)**.

ARTICLE

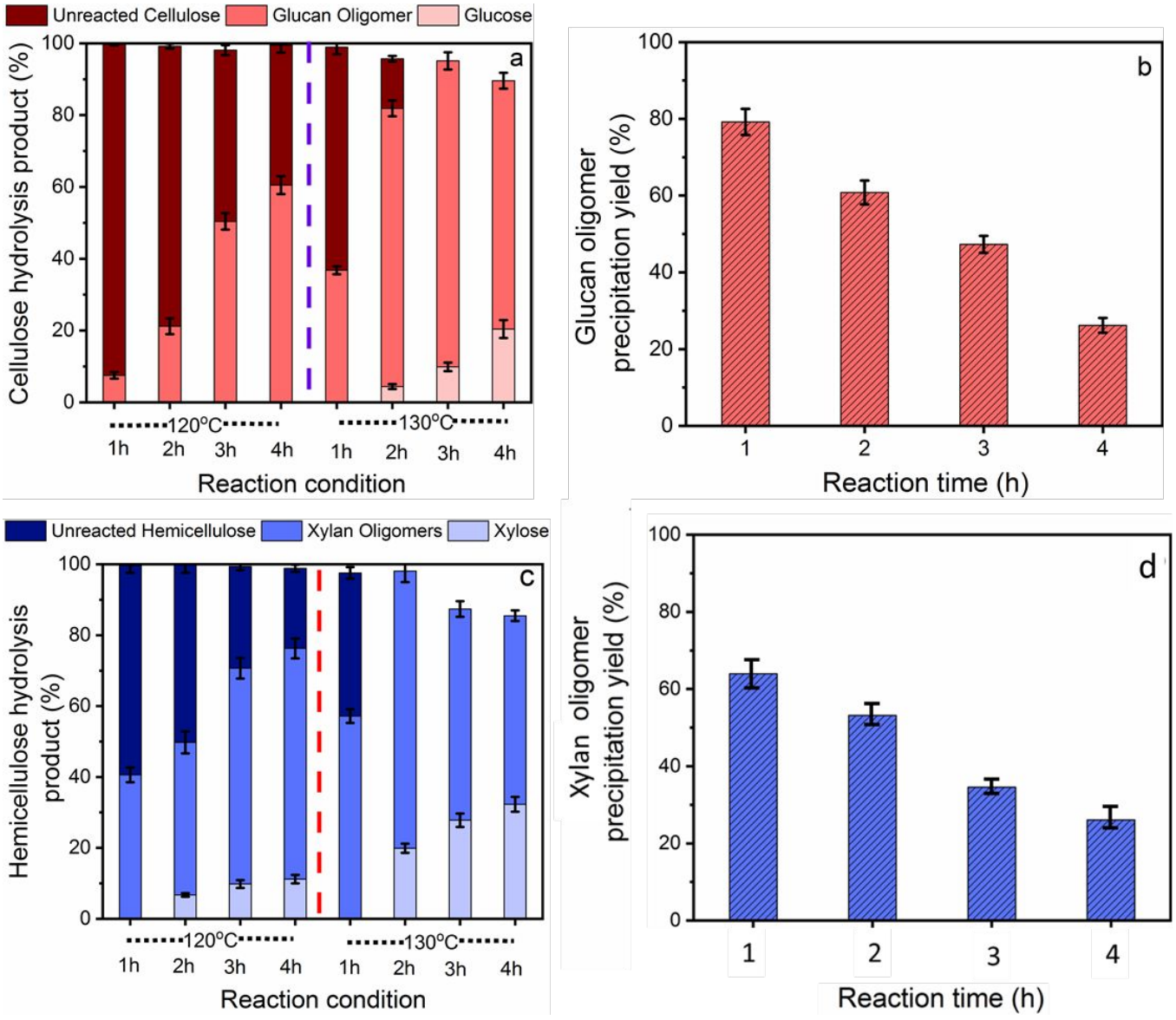


Figure 6. Hydrolysis of cotton straw in the MSH and precipitation of glucan oligomers from the hydrolysates. (a) Product distributions obtained from the hydrolysis of cellulose of cotton straw in the MSH. (b) Precipitation yield of glucan oligomer hydrolyzed at 130 °C with MeOH addition. (c) Product distributions obtained from the hydrolysis of hemicellulose of cotton straw in the MSH. (d) Precipitation yield of xylan oligomer hydrolyzed at 130 °C with MeOH addition. Reaction conditions: MSH hydrolysis: 300 mg of biomass, 6 g of MSH; Precipitation: 1 g of hydrolysate, 10 g of MeOH.

Recycle of MSH after precipitation

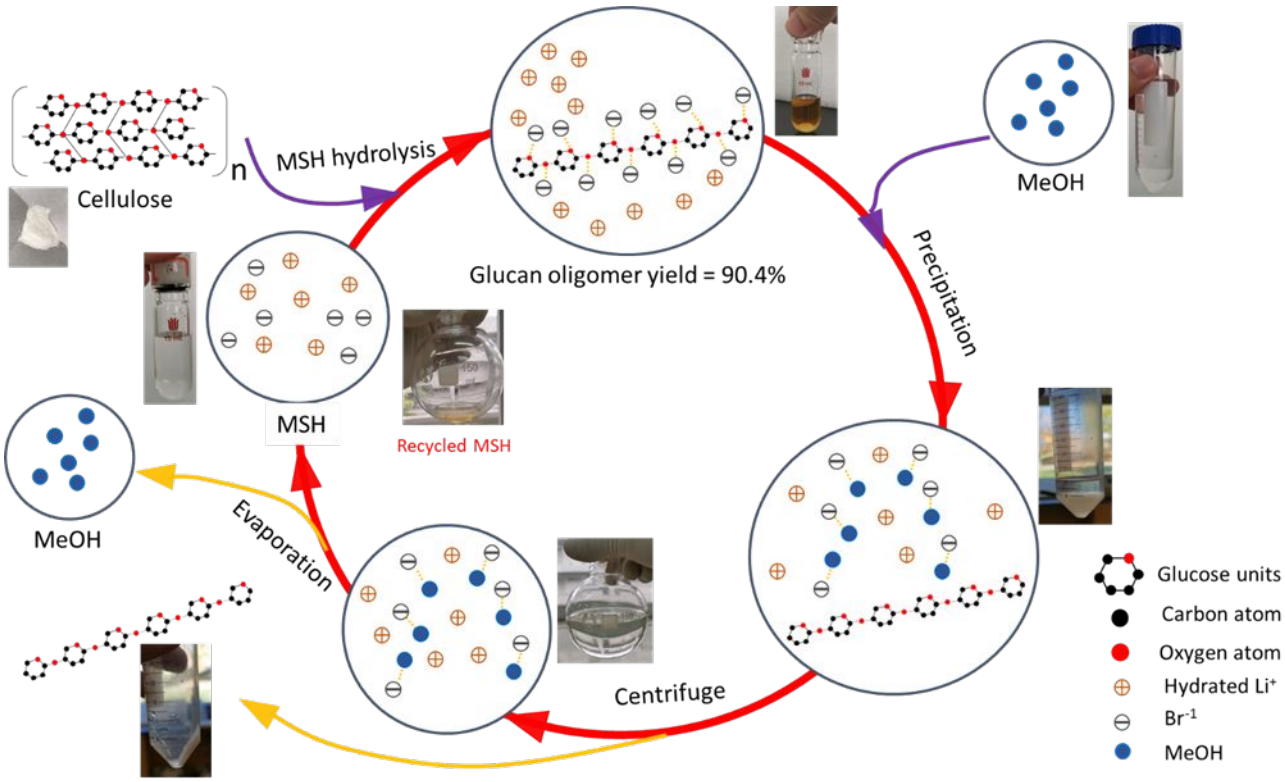
After the precipitation of the formed glucan oligomers with MeOH addition and filtration, MeOH in the MSH solvent was removed with rotary evaporation at 60 °C for 1 h. The recycled MSH was used again for the hydrolysis microcrystalline cellulose. The hydrolysates and oligomer yield before and after recycling two times were shown in **Figure S4**. It was found that the hydrolysates changed from transparent yellow solution to turbid solution and then to gel with oligomer yield decreased from 90.4% to 73.5% and then to 39.7% during the recycle process. The largely reduced oligomer yield may be because of the by-products formed during the hydrolysis, which includes 5-HMF, formic acid, levulinic acid, et al (shown in **Table S2**).

The organic components, in particular, 5-HMF and LA can facilitate the further hydrolysis of glucan oligomer into glucose and formation of humins.[47, 48] In order to fully recycle the used MSHs, a purification process for removing the compounds such as selective adsorption is required.

Conclusion

Separation of the sugars from ionic liquid, deep eutectic solvent and MSHs is one of the major challenges for applying the solvents in hydrolysis of lignocellulose biomass. In this study, we demonstrate that compared to glucose it is more efficient to separate glucan oligomers from the MHSs using MeOH as an anti-solvent. Based on the discovery, a stepwise process was introduced to selectively convert crystalline cellulose and cotton straw into monosaccharide, as shown in **Scheme 1**. In the process, crystalline cellulose was first hydrolyzed into glucan oligomers in the MSH of LiBr with a yield as high as of 90.4%. The produced glucan oligomers were precipitated out from the MSHs by adding MeOH as an anti-solvent. It was found that the precipitation efficiency was affected by the solubility of the glucan oligomers in the anti-solvent and MSHs. MeOH as an anti-solvent can precipitate the glucan oligomer as the concentration as low as 1.64 wt% with a yield of 90.3%. However, glucose is highly soluble in the MSH and cannot be precipitated using the anti-solvent precipitation method. The precipitation efficiency increases with the chain length of the glucan oligomers. Oligomers with β(1→4) linkages are easier to precipitate than branched oligomers with α(1→4) linkages. The precipitated oligomers can be further hydrolyzed into glucose with a yield of 99.7%. The method can be also used for selective production of glucan oligomer and xylan oligomers from raw biomass. The technique provides an efficient way to hydrolyze cellulose and separate produced oligomers even at low sugar concentration of 1.64 wt.%. The obtained oligomer can be selectively converted into monosaccharide or might be used in other emerging applications such as healthcare and agriculture.[49-51]

Scheme 1. The stepwise process for hydrolyzing cellulose into glucan oligomers in MSHs followed by separation using MeOH as an anti-solvent.



Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Qiyu Liu, Xinping Ouyang and Xueqing Qiu acknowledge the supports from the National Key Research and Development Program of China (2019YFC1905301), National Natural Science Foundation of China (22008078) and the National Natural Science Foundation of China (22078115). The visit of Qiyu Liu to UMass Amherst was supported by Guangdong Outstanding Research Talents International Visiting Program (In 2019). The experiments were carried out at UMass Amherst and supported by the Catalysis Center for Energy Innovation, an Energy Frontier Research Center funded by the US Department of Energy, Office of Science, and Office of Basic Energy Sciences under award number DE-SC0001004.

Notes and references

1. G. W. Huber, S. Iborra and A. Corma, *Chem. Rev.*, 2006, **106**, 4044-4098.
2. P. Gallezot, *Chemical Society Reviews*, 2012, **41**, 1538-1558.
3. H. Chen, J. Liu, X. Chang, D. Chen, Y. Xue, P. Liu, H. Lin and S. Han, *Fuel Processing Technology*, 2017, **160**, 196-206.
4. Y.-B. Huang and Y. Fu, *Green Chemistry*, 2013, **15**, 1095-1111.
5. N. Sathitsuksanoh, A. George and Y. H. P. Zhang, *Journal of Chemical Technology & Biotechnology*, 2013, **88**, 169-180.
6. C. G. Yoo, Y. Pu and A. J. Ragauskas, *Current Opinion in Green and Sustainable Chemistry*, 2017, **5**, 5-11.
7. R. Rinaldi, R. Palkovits and F. Schüth, *Angewandte Chemie*, 2008, **120**, 8167-8170.
8. J. A. Sirviö, M. Visanko and H. Liimatainen, *Biomacromolecules*, 2016, **17**, 3025-3032.
9. S. Sen, J. D. Martin and D. S. Argyropoulos, *ACS Sustainable Chemistry & Engineering*, 2013, **1**, 858-870.
10. N. Rodriguez Quiroz, A. M. Padmanathan, S. H. Mushrif and D. G. Vlachos, *ACS Catalysis*, 2019, **9**, 10551-10561.
11. Y. Chen, X. Zhang, T. You and F. Xu, *Cellulose*, 2019, **26**, 205-213.
12. S. Park and R. J. Kazlauskas, *Current Opinion in Biotechnology*, 2003, **14**, 432-437.
13. D. J. van Osch, L. J. Kollau, A. van den Bruinhorst, S. Asikainen, M. A. Rocha and M. C. Kroon, *Physical Chemistry Chemical Physics*, 2017, **19**, 2636-2665.
14. W. Deng, J. R. Kennedy, G. Tsilomelekis, W. Zheng and V. Nikolakis, *Industrial & Engineering Chemistry Research*, 2015, **54**, 5226-5236.
15. N. Rodriguez Quiroz, A. M. Norton, H. Nguyen, E. Vasileiadou and D. G. Vlachos, *ACS Catalysis*, 2019, **9**, 9923-9952.
16. J. van den Bergh, W. Wiedenhof, D. Siwy and H. Heinerman, *Adsorption*, 2017, **23**, 563-568.
17. T. Youngs, C. Hardacre and J. Holbrey, *The Journal of Physical Chemistry B*, 2007, **111**, 13765-13774.
18. M. Tian and K. H. Row, *Journal of chromatographic science*, 2013, **51**, 819-824.
19. X. Pan and L. Shuai, *Journal*, 2015.
20. Q. Liu, Q. Ma, S. Sabnis, W. Zheng, D. G. Vlachos, W. Fan, W. Li and L. Ma, *Green Chemistry*, 2019, **21**, 5030-5038.
21. Y. He, D. M. Bagley, K. T. Leung, S. N. Liss and B.-Q. Liao, *Biotechnology advances*, 2012, **30**, 817-858.
22. A. Gautam and T. J. Menkhaus, *Journal of Membrane Science*, 2014, **451**, 252-265.
23. C. Li and Z. K. Zhao, *Advanced Synthesis & Catalysis*, 2007, **349**, 1847-1850.
24. A. S. Amarasekara and O. S. Owereh, *Industrial & Engineering Chemistry Research*, 2009, **48**, 10152-10155.
25. F. Tao, H. Song and L. Chou, *Bioresource technology*, 2011, **102**, 9000-9006.
26. H. Ren, R. Gong, M. Li, Y. Liu, H. Zhu, C. Wang and E. Duan, *Journal of Molecular Liquids*, 2020, **312**, 113282.
27. J. van den Bergh, I. V. Babich, P. O'Connor and J. A. Moulijn, *Industrial & engineering chemistry research*, 2017, **56**, 13423-13433.
28. P. Yu, W. Hung and H. Wan, *Journal of the Taiwan Institute of Chemical Engineers*, 2018, **93**, 193-200.
29. S. Sadula, O. Oesterling, A. Nardone, B. Dinkelacker and B. Saha, *Green Chemistry*, 2017, **19**, 3888-3898.
30. J. Wang, H. Cui, Y. Wang, R. Zhao, Y. Xie, M. Wang and W. Yi, *Green Chemistry*, 2020, **22**, 4240-4251.
31. M. Lara-Serrano, S. Morales-delaRosa, J. M. Campos-Martín and J. L. Fierro, *Green Chemistry*, 2020, **22**, 3860-3866.
32. N. Li, Z. Wang, T. Qu, J. Kraft, J.-H. Oh, J.-P. van Pijkeren, G. W. Huber and X. Pan, *Green Chemistry*, 2019, **21**, 2686-2698.
33. P. Chen, A. Shrotri and A. Fukuoka, *ChemSusChem*, 2019, **12**, 2576-2580.
34. P. Chung, M. Yabushita, A. T. To, Y. Bae, J. Jankolovits, H. Kobayashi, A. Fukuoka and A. Katz, *ACS Catalysis*, 2015, **5**, 6422-6425.
35. P. Dornath, H. J. Cho, A. Paulsen, P. Dauenhauer and W. Fan, *Green Chemistry*, 2015, **17**, 769-775.
36. P. Dornath, S. Ruzycky, S. Pang, L. He, P. Dauenhauer and W. Fan, *Green Chemistry*, 2016, **18**, 6637-6647.
37. R. Rinaldi, R. Palkovits and F. Schüth, *Angewandte Chemie International Edition*, 2008, **47**, 8047-8050.
38. L. A. Alves, J. B. Almeida e Silva and M. Giulietti, *Journal of Chemical & Engineering Data*, 2007, **52**, 2166-2170.
39. L. Wang, S. Chen, T. Shu and X. Hu, *ChemSusChem*, 2019.
40. C. de Azevedo Souza, S. Li, A. Z. Lin, F. Boutrot, G. Grossmann, C. Zipfel and S. C. Somerville, *Plant physiology*, 2017, **173**, 2383-2398.
41. E. Billès, V. Coma, F. Peruch and S. Grelier, *Polymer International*, 2017, **66**, 1227-1236.
42. W. Li, Q. Liu, Q. Ma, T. Zhang, L. Ma, H. Jameel and H.-m. Chang, *Bioresource technology*, 2016, **219**, 753-756.
43. Q. Liu, W. Li, Q. Ma, S. An, M. Li, H. Jameel and H-m. Chang, *Bioresource technology*, 2016, **211**, 435-442.
44. A. Sluiter, B. Hames, D. Hyman, C. Payne, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton and J. Wolfe, *National Renewable Energy Laboratory*, 2008, **9**.
45. A. Shrotri, L. K. Lambert, A. Tanksale and J. Beltramini, *Green chemistry*, 2013, **15**, 2761-2768.
46. K. M. Hello, H. R. Hasan, M. H. Sauodi and P. Morgen, *Applied Catalysis A: General*, 2014, **475**, 226-234.
47. N. Shi, Q. Liu, H. Cen, R. Ju, X. He and L. Ma, *Biomass Conversion and Biorefinery*, 2020, **10**, 277-287.
48. Z. Xu, Y. Yang, P. Yan, Z. Xia, X. Liu and Z. C. Zhang, *RSC Advances*, 2020, **10**, 34732-34737.
49. N. Li, Y. Li, C. G. Yoo, X. Yang, X. Lin, J. Ralph and X. Pan, *Green Chemistry*, 2018, **20**, 4224-4235.
50. B. K. Knapp, L. L. Bauer, K. S. Swanson, K. A. Tappenden, G. C. Fahey and M. R. De Godoy, *Nutrients*, 2013, **5**, 396-410.
51. T. Hasunuma, K. Kawashima, H. Nakayama, T. Murakami, H. Kanagawa, T. Ishii, K. Akiyama, K. Yasuda, F. Terada and S. Kushibiki, *Anim. Sci. J.*, 2011, **82**, 543-548.