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A new route to high yield sugars from biomass: phosphoric–sulfuric acid

Mark A. Harmer,*[a] Analine Fan,[b] Ann Liauw[a] and Ravinash Krishna Kumar[c]

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We have developed a simple and effective route for the high yield extraction of sugars from cellulosic based biomass. This process uses a combination of a cellulose decrystallization step with a mixture of phosphoric and sulfuric acid, followed by a hydrolysis step producing sugars (xylose and glucose) with yields of approximately 90%.

The industrialized world's unchecked depletion of crude oil reserves is set to result in a future global shortage. At the same time, the world's supply of untapped oil fields will certainly be inadequate to meet its needs. Finding a solution to the consequent shortage of petroleum based fuels and chemicals is one of the most important challenges that this generation of scientists will face. Replacing such materials with 'biofuels' and 'bio-based' chemicals will be a major part of this solution.[1]

Corn stover is the largest single source of biomass in the United States and has the advantage of being almost ubiquitously available. Other types of cellulosics include bagasse, switch-grass to wood based materials. The challenge we will face is the effective collection and conversion of a range of biomass sources. We will describe a new route to high yield sugars, an important step in the production of sustainable fuels and chemicals for the future.

The chemical and enzymatic extraction of sugars from lignocellulosic biomass include the use of dilute acid treatments at elevated temperatures and pressures,[2–4] the combinations of cellulose pre-treatments followed by enzymatic hydrolysis[5] and use of a concentrated acid decrystallization followed by a lower acid concentration hydrolysis.[6,7] There is still a need, however, for improved processes to obtain high yields of sugars from biomass at a reasonable cost.

The use of a dilute acid has been shown to produce high yields of xylose, however the yield of glucose is very low (5–10%).[2] This is due to the recalcitrant nature of the highly crystalline cellulose. Higher yields of glucose have been achieved using a two step acid hydrolysis process, a lower temperature step to remove the xylose and then a subsequent higher temperature hydrolysis (around 220 °C)[3,4]. The reported yields of glucose and xylose were around 57% and 84%, respectively. All of these approaches require high pressures, multiple steps and complicated and expensive equipment. Biomass loadings in the reactor also tend to be quite low for practical purposes (5–12 wt%). In order to obtain high yields of glucose the importance of decrystallization of cellulose has been demonstrated for many years using >65 wt% sulfuric acid as the decrystallization agent.[6,7] A seminal paper in this area was published as early as 1933, with citations dating back to 1827.[6] Yields of glucose were around 80%. These methods can lead to considerable charring due to the degradation of xylan leading to low yields of xylose. A small scale facility using this approach has been developed and adopted by the company Bluefire.[7] Ionic liquids have also been investigated, in combination with acid catalysis, to study the hydrolysis of lignocellulosic biomass.[8] A number of interesting reports on the use of solid acid catalysts have recently been described.[9–13] In general the yield of glucose is low. It was shown however that a combination of extended ball milling, which reduces crystallinity, improves the glucose yield to around 40%.

The combination of chemical pre-treatments with enzyme hydrolysis has recently been reviewed.[5,14] The costs of the enzymes are quite high and the activity is very dependent upon the biomass source.

We have developed a new route to high yield sugars from biomass based upon the use of a mixture of phosphoric acid and sulfuric acid for the decrystallization step, followed by dilution with water to carry out the hydrolysis step. The hydrolysis of glucan and xylan produce glucose and xylose, respectively. For a biomass source, we used corn cob which was milled to about 2 mm. This was analyzed using the NREL method[15] to establish the exact xylan and glucan content. The xylan and glucan content was determined to be 28.1 and 35.26 g per 100 g of dry cob.

In our initial experiments we carried out the hydrolysis of corn cob in the presence of 10 wt% sulfuric acid alone, for comparison, at 90 °C (a biomass loading of 10 wt%). The results are shown in Fig. 1. This confirmed what is known in the literature—that reasonably high xylose yields are obtained (75–85%) although the glucose yield is very low (less than 10%).

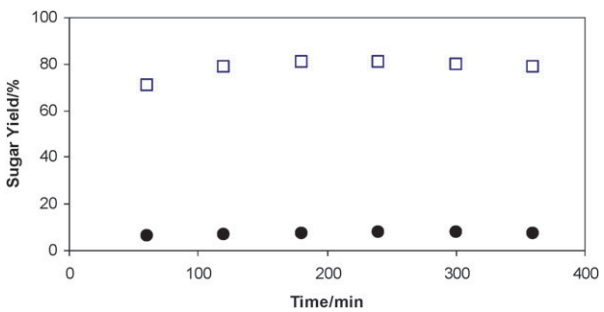

Fig. 1 Xylose (□) and glucose (●) yield *vs.* time without decrystallization.

[a] *DuPont Central Research and Development, Experimental Station, Wilmington, DE 19880, USA. E-mail: mark.a.harmer@usa.dupont.com*
[b] *School of Chemistry, University of Edinburgh, UK*
[c] *School of Chemistry, University of Bristol, UK*

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The yield of glucose can be increased by decrystallizing the cellulose using concentrated sulfuric acid. Under a wide range of conditions the highest attainable yield of glucose was around 75–80% with 70% xylose. The main problem with sulfuric acid is that the reaction is very difficult to control. In a typical experiment 10 g of corn cob was added to 15 g of acid (>72%). The mixture was slowly stirred for about 15–30 min. Longer and shorter times were investigated resulting in lower yields. The decrystallization could be followed using a polarizing microscope, which showed the optimum decrystallization time was between 15 and 30 min. Water was then added to yield a solution with a biomass content of about 10% and an acid loading of 15%. It was difficult to use higher biomass concentrations (for example 20 wt% biomass) since the resulting acid concentration during hydrolysis was high leading to xylose decomposition. Furthermore, the reaction of the pure acid is exothermic when mixed with the biomass and is difficult to control on larger scale. Using this approach the optimum yield of xylose and glucose (heating to 85 °C) was around 70 and 75%, respectively.

To obtain higher sugar yields we have developed a new, simple and yet very effective route to the extraction of sugars from biomass. A mixture of sulfuric acid with phosphoric acid appears to be an ideal decrystallization agent for biomass. We have looked at mixtures in the range of phosphoric–sulfuric acid from 40 : 60 to 70 : 30. Over this range, decrystallization occurs very readily at room temperature. The mixed acid system provides an excellent balance for rapid and controllable decrystallization without biomass degradation.

The extent of decrystallization was measured using a Nikon polarizing microscope, Eclipse LV100POL and X-ray. Fig. 2 shows a polarizing microscope image for corn cob (about 0.2 mm in size) in the presence of a mixture of phosphoric and sulfuric acid. The images shown are at 5 min (upper left) and 30 min (upper right) in a 50 : 50 mixture. The crystalline particles appear white and darken upon decrystallization. After about 30 min the corn cob is almost completely decrystallized (>95%). The decrystallization time can also be controlled by varying the phosphoric acid to sulfuric acid ratio. Fig. 2 (lower left) shows the controlled decrystallization of corn cob using a 70 : 30 phosphoric–sulfuric mixture, showing partial decrystallization, after 90 min. Note the substructural detail within the corn cob. Fig. 2 (lower right) shows a fully decrystallized sample of corn cob after 4 h, viewed using non-polarized light. An equivalent sulfuric acid route is black and significantly degraded however the phosphoric–sulfuric based material is light brown. By varying the ratio of the two acids the decrystallization can be controlled from minutes (very high sulfuric acid) to days (very high phosphoric).

The reduction in crystallinity is also revealed using X-ray diffraction. The original corn cob shows distinct crystalline peaks (16, 22 and 34.5, 2θ) corresponding to the 101, 002 and 040 planes, respectively, for crystalline cellulose. These are reduced to a broad peak following decrystallization.[16] Interestingly the decrystallized cellulose shows an appreciable solubility in water (up to 50% of the biomass). Gel phase chromatography of the soluble component shows a broad band with a molar mass (g mol^{-1}) of between 1000 and 10 000 in water, and a mean of around 5000. The decrystallized cellulose was also found to contain around 1.1 wt% of P and 1.05 wt% S (based upon dry weight) which is consistent with the formation of sulfate and phosphate esters. Presumably, during decrystallization, some chain hydrolysis occurs leading to a partially substituted long chain oligomer. The sulfates and phosphates are removed (cleaved) upon hydrolysis.

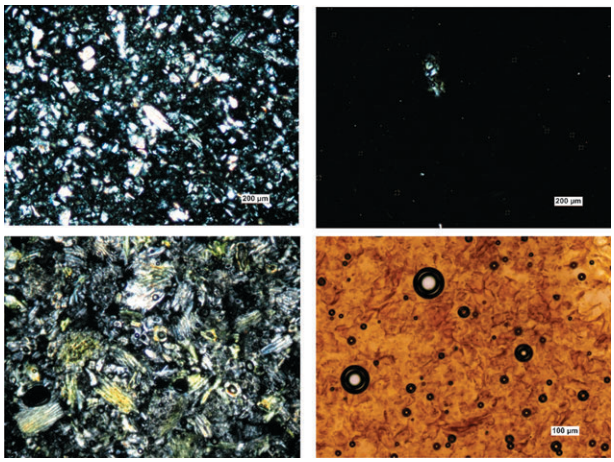

Fig. 2 Optical micrographs showing the decrystallization of corn cob using a mixture of sulfuric acid and phosphoric acid, view using polarized light except for lower right.

In a typical sugar extraction, 100 g of corn cob (particle size about 2 mm) were added to 200 g of a 70 : 30 mixture of phosphoric acid (originally 85%) and sulfuric acid (95–98%). This was slowly mixed in a KitchenAid blender (keeping the temperature below 30 °C by slow stirring). The initial thick mixture was left to decrystallize for 16 hours. This produces a paste like material which showed little to no crystallinity. Water (200 g) was then added and the material was heated at 80 °C and samples were taken every hour. The biomass loading was 20 wt% and the resulting sulfuric concentration was 12%. The sample was diluted with water (20-fold) in order to get an accurate sugar content *via* HPLC and acid was removed *via* basic anion exchange. The HPLC used was a Waters Alliance 2695 Separation Module with 2414 Refrac Index Detector, running in 0.01 N sulfuric acid (aq.) through an Aminex HPX087H 30 mm × 7.8 mm column at 50 °C with a rate of 6 mL min^{-1}. Standards were used to calibrate the column. The results are shown in Fig. 3.

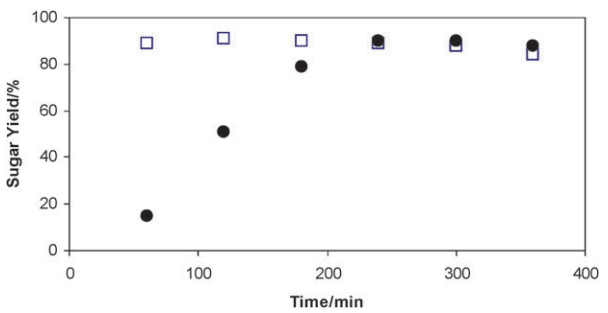

Fig. 3 Xylose (□) and glucose (●) yields *vs.* time following decrystallization.

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The yield of xylose was about 90% and the glucose yield reached 90% after about four hours of hydrolysis. As far as we are aware these are the highest yields reported from both glucose and xylose *via* a single hydrolysis step, under atmospheric pressure and less than 100 °C. It is also interesting to note that the xylose yield is higher than in the absence of a decrystallization step, which is consistent with at least some of the xylan being tied up within the crystalline glucan structure. We also detected close to about 10% cellobiose (the dimer of glucose), which accounts for an almost quantitative yield of glucan hydrolysis. A small amount of furfural (less than 0.8% based upon total sugars) and hydroxymethylfurfural, HMF, (less than 0.08%) was also detected. The yield of the sugars was also determined at the end of the reaction by removing the acid *via* ion exchange, concentrating the sugars and measuring the yields. The NMR showed the expected spectra for a combined xylose and glucose sugar extraction. We have investigated a wide range of reaction conditions; for example, we have varied the ratio of acid from 70 : 30 to 50 : 50 to 60 : 40, and similar results were found with each of these. Particle size did have an effect. For smaller particle sizes, the time for decrystallization was less. For example, if the 2 mm particle was ground to about 0.5 mm, then decrystallization was complete within about three hours.

One of the remarkable features of this method is the apparent stability of the biomass in the acid mixture. We found that the biomass was stable (*i.e.*, we could obtain 90% sugar yields) after leaving it in contact with concentrated acid for up to three days, after which degradation begins to occur. Leaving biomass in contact with sulfuric for an equivalent time reduces the xylose yield to about 50%. The use of a lower inherent sulfuric acid concentration (essentially diluted down by a weaker acid, phosphoric) should also be safer to handle than neat sulfuric. The mixed acid system is more versatile than the pure sulfuric acid based system. A higher biomass loading (at least 22 wt%) is easy to attain. The mixed acid approach was also very effective with other types of biomass including switchgrass, bagasse and milled pine. In the case of bagasse, yields of about 80–90% glucose and xylose were readily obtained (also at a high biomass loading of about 20 wt%). Switchgrass (about 1 mm in size) was also treated with a 70 : 30 mixture of the phosphoric–sulfuric, for 24 hours, water added to about 20 wt% biomass and then hydrolyzed at 83 °C for up to 5 hours. The yield of sugars was about 76% xylose and 80% glucose. We also carried out some initial fermentation experiments, fermenting the sugars to ethanol. The conversion was very similar to enzyme derived sugars, at similar yields (details not given), demonstrating the approach used does not appear to introduce any inhibitors that interfere with ethanol production. A more detailed account of this will be published separately.

In summary, we have developed a new and very simple decrystallization route for biomass using a combination of sulfuric with phosphoric acid. The mixture allows decrystallization of biomass without significant degradation. The total time for decrystallization can vary from minutes to 3 days depending upon the ratio. Subsequent hydrolysis leads to 90% sugar yields. This new method also lends itself generally for the processing of biomass for a range of applications.

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