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Fractionation of lignocellulosic biomass with the ionic liquid 1-butylimidazolium hydrogen sulfate

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The application of the protic ionic liquid 1-butylimidazolium hydrogen sulfate in the deconstruction (aka pretreatment) and fractionation of lignocellulosic biomass has been investigated. A cellulose rich pulp and a lignin fraction were produced. The pulp was subjected to enzymatic saccharification which allowed recovery of up to 90% of the glucan as fermentable glucose. The influence of the solution acidity on the deconstruction of *Miscanthus giganteus* was examined by varying the 1-butylimidazole to sulfuric acid ratio. Increased acidity led to shorter preteatment times and resulted in reduced hemicellulose content in the pulp. Addition of water to the ionic liquid resulted in enhanced saccharification yields. The ability to tune acidity through the use of protic ionic liquids offers a significant advantage in flexibility over dialkylimidazolium analogues.

Introduction

Wide-spread use of petroleum as a feedstock for transportation fuels and chemicals during the last century has provided low-cost energy and materials but also contributed to a historic high in atmospheric carbon dioxide concentrations. The anticipated depletion of petroleum reserves in the next few decades has led to intense interest in the use of plant biomass as the next generation feedstock for both fuels and chemical products.

Bioethanol produced from sucrose (sugar cane) or starch (cereals such as corn) is already increasingly used as large-scale substitute transportation fuel but has been criticised for direct competition with food production[1] and indirect carbon dioxide emissions due to land use change.[2]

To mitigate this, lignocellulosic biomass could be used. It is the most abundant plant material on the planet and comprises a variety of agricultural residues and forest crops, municipal waste and dedicated biofuel crops. 60-70 wt% of the lignocellulosic biomass are fermentable carbohydates, stored as cellulose and hemicellulose. The remainder is lignin and a number of minor components such as extractives and inorganic compounds. The use of lignocellulosic biomass for the provision of fermentable carbohydrates is more challenging than the use of sucrose and starches due to the distinct architecture of woody plant tissues.[3] No pretreatment method is currently capable of achieving lignocellulose deconstruction in an economically viable commercial scale process.

A possible deconstruction strategy for lignocellulose is the separation of two of the major components, cellulose and lignin. The cellulose fraction can be hydrolysed to glucose and fermented, while lignin may serve as a source of aromatic chemicals.

Ionic liquids (ILs) are salts that melt at low temperature and have attracted a great deal of interest.[4] An increasing number of studies have sought to apply these liquids to the deconstruction of lignocellulosic biomass.[5] Potential advantages over other methods are the ability of certain ionic liquids to dissolve/decrystallise cellulose (Dissolution Process) and/or to extract and dissolve lignin (Ionosolv process).

It has been shown that the ability of an ionic liquid to dissolve lignocellulose components is mostly determined by the nature of its anion, with the cation having a secondary effect. We have previously shown that the ionic liquid 1-butyl-3-methylimidazolium hydrogen sulfate, $[C_4C_1im][HSO_4]$, is very effective in the delignification of a variety of lignocellulosic feedstocks, including the high yielding perennial grass *Miscanthus giganteus*.[6]

The synthesis of 1,3-dialkylimidazolium ionic liquids requires alkylation of a 1-alkylimidazole, often followed by anion metathesis.4 These additional synthesis steps are responsible for the high cost of ionic liquids, the most common criticism of the use of ionic liquids for biomass processing.[7]

The substitution of dialkylimidazolium ionic liquids ($[C_nC_mim][anion]$) with 1-alkylimidazolium ionic liquids ($[C_nHim][anion]$) formed by simple acid-base chemistry has the potential to greatly reduce the cost and environmental impact of ionic liquid production. Initial experiments using 1-butylimidazolium hydrogen sulfate, $[C_4Him][HSO_4]$, for the deconstruction of lignocellulosic biomass resulted in extensive delignification and hemicellulose removal, comparable to its dialkylated counterpart.[6] We therefore decided to investigate the use of this ionic liquid in greater detail.

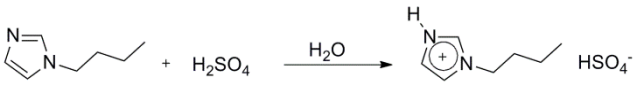
Our previous results also suggested that addition of a certain amount of water is likely to increase performance, therefore mixtures of the ionic liquid with water were

investigated. Other preliminiary data showed that the outcome of the pretreatment varied with ionic liquid batch, suggesting that control of the acid:base ratio during the preparation of $[C_4Him][HSO_4]$ may be an important variable. We therefore chose to study this aspects in more depth and report the results of these investigations here.

Results and Discussion

Ionic liquid synthesis

1-butylimidazolium hydrogen sulfate was prepared by mixing 1-butylimidazole (C_4im) and sulfuric acid in various ratios according to Scheme 1. The ionic liquid (1:1 mixture) was a viscous liquid at room temperature.



Scheme 1 Synthesis of 1-butylimidazolium hydrogen sulfate, $[C_4Him][HSO_4]$.

We have chosen to depict the proton transfer equilibrium being completely on the product side. However, it must be considered that proton transfer from acid to base may be incomplete, resulting in tertiary mixtures (acid, base and salt). Incomplete proton transfer can be observed for example when amines are combined with weak acids such as acetic acid.[8] This will affect the physical properties of the ionic liquid (such as conductivity). In addition, if either of the neutral components in such ionic liquids have sufficient volatility at the desired operating temperature, it will also result in solvent emissions and potential changes in the composition during the process.

Angell and co-workers proposed to use electrical conductivity as a measure for completeness of the proton transfer.[9] They correlated the ionic liquids' electrical conductivity with ΔpK_a, the difference of the standard pK_a values for the acid and the base's conjugate acid (measured in dilute aqeuous solution). They concluded that a ΔpK_a larger than 10 would result in complete proton transfer, as conductivity for such ion combinations was the same as for aprotic ionic liquids.

The pK_a of sulfuric acid is -3.0 while it is ~7.0 for 1-butylimidazolium. This appears to be a sufficiently large ΔpK_a to assume full proton transfer.

Table 1 1-Butylimidazolium hydrogen sulfate ionic liquid solutions used in this study, their $C_4Im:H_2SO_4$ ratio (molar ratio), the yield and water content after drying the ionic liquid.

$C_4Im:H_2SO_4$	Yield (%)	H_2O (wt%)
1.00:1.50	99	0.89
1.00:1.01	96	0.33
1.00:0.99	98	0.87
1.00:0.80	98	1.60
1.00:0.67	99	2.83
1.00:0.50	99	2.33

Scheme 1 depicts the synthesis of an ionic liquid resulting from a 1:1 mixture of the starting materials. It is also possible to add an excess of either acid or base. Excess acid will result in the presence of undissociated sulfuric acid, while excess base could result in formation of $[C_4Him]_2[SO_4]$ and/or the presence of unprotonated 1-butylimidazole.

In this paper, we will refer to all ionic liquids by their molar acid:base ratio ($C_4im:H_2SO_4$). The ratios used in this study are listed in Table 1. We will also specify the ionic liquid content in aqueous solutions with a subscript where necessary. For instance, a mixture consisting of 80 vol% $C_4im:H_2SO_4$ = 1.00:0.99 and 20 vol% H_2O will be labelled as $[C_4im:H_2SO_4] = [1.00:0.99]_{80\%}$.

It should be noted that the substantial acidity of the $[HSO_4]^-$ anion (pK_a of hydrogen sulfate is ~2) results in all the aqueous compositions used in the study possessing a pH<7, even those with an excess of 1-butylimidazole.

The ionic liquid synthesis by combining an acid and a base is straightforward, however, care must be taken to use the correct amount of acid and base in order to make valid comparisons between to mixtures. We have endavoured to control the acid base ratio by careful dosing of sulfuric acid and 1-butylimidazole based on the purity stated by the manufacturer. However, since purity of both acid and base was <100%, we cannot guarantee that the mixtures used in this study had the exact compositions we aimed for (up to 4% deviation possible). However, the relative acidity of the compositions is consistent and so the trends we observed are valid.

Biomass deconstruction

Ground *Miscanthus* was subjected to the processing sequence detailed in Figure 1. It was first treated with the ionic liquid solutions and corresponding aqueous mixtures listed in Table 1 without stirring at 120°C for 15 min up to 24 h. After the treatment, the ionic liquid solutions were diluted with the short-chain alcohol methanol. The undissolved solids (pulp) were separated from the liquid, washed using methanol (which dissolves the ionic liquid and does not act as an antisolvent for lignin) and air-dried. The methanol also removes other solutes such as 5-hydroxymethyl furfural, furfural and organic acids. Part of the pulp was subsequently subjected to enzymatic saccharification, while another part was used to determine the composition of the pulp (glucose, hemicellulose, lignin and ash content).

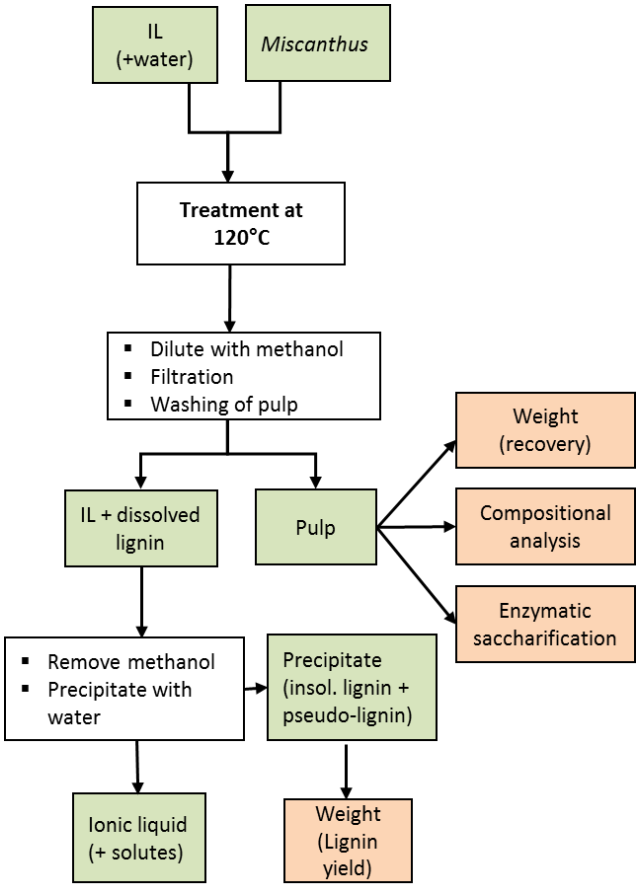


Figure 1 Process flow for deconstructing lignocellulosic biomass in this study

The ionic liquid liquor was reconcentrated and diluted with water in order to induce precipitation of a lignin rich fraction. The precipitate was separated from the ionic liquid by centrifugation, washed, dried and its weight determined.

Pulp recovery

For this study we measured the yield of pulp relative to the biomass. A complex combination of effects was observed. For some treatment conditions pulp recoveries in excess of 100% were achieved. The highest recovery, 128%, was observed for the dry ionic liquid solution with 50% excess sulfuric acid employed for 24 h. The cause of the excess recovery is contamination of the pulp with ionic liquid that was not removed during washing, which will be discussed in more detail below. Less pulp was recovered after treatment with ionic liquid water mixtures than after treatment with dry IL solutions, indicating reduced ionic liquid contamination of the pulp. Pulp recovery also decreased with increasing IL acidity and extended treatment time. The lowest yield, 41%, was obtained after deconstruction with C_4im:H_2SO_4 = $[1.00{:}1.01]_{80\%}$ after 24 h. As we will demonstrate in the section on pulp composition, these lowest yields were due to extensive removal of hemicellulose and lignin but also partial hydrolysis of the cellulose.

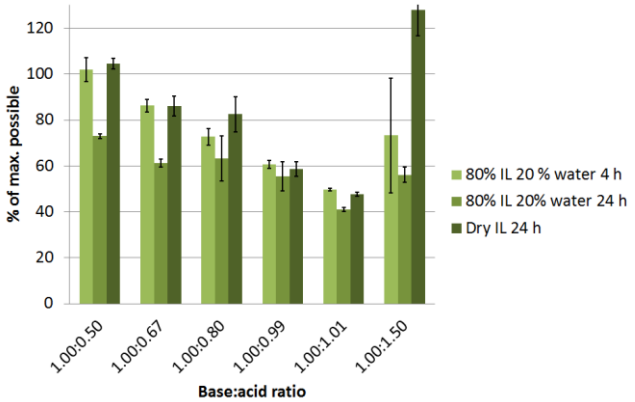


Figure 2 Biomass recovery in wt% after deconstruction with C_4im:H_2SO_4 and the $[C_4\text{im}{:}H_2SO_4]_{80\%}$ solutions at 120 °C.

Effect of the IL acidity on the pulp composition

The quantities of lignin, hemicellulose and cellulose in the pulps were determined according to NREL standard protocols.[10] The mass balance was calculated by summing the mass fraction (in %) of all components (glucan, xylan, arabinan, galactan, mannan, lignin, ash content and extractives). The sum of the individual components should be close to 100% (the mass balance rarely matches exactly 100% due to small imperfections in the method). However, we observed deviations in our experiments that were larger than expected. In some cases, the components only accounted for 80% of the pulp yield (Figure 3). The mass balance was particularly poor after deconstruction with dry C_4im:H_2SO_4.

Explanation for the poor mass closure came from washing experiments. It was observed that modifying the original procedure with an extra washing step resulted in better mass balance, supporting our proposition that the additional mass recovered in the pulp was likely to caused by ionic liquid. The additional washing step was introduced in the deconstruction procedure for C_4im:H_2SO_4 = 1.00:1.01 (both aqueous and water-free) and led to mass balances of *ca*. 99%.

Although the presence of ionic liquid in the pulp could be a problem for various reasons, for example the deactivation of enzymes during saccharification, it is not detrimental to the accuracy of the compositional analysis. After taking into account the presence of ionic liquid in the pulp, we were able to obtain compositional data that were consistent. They are given in Table 2 and visualised in Figures 4-6.

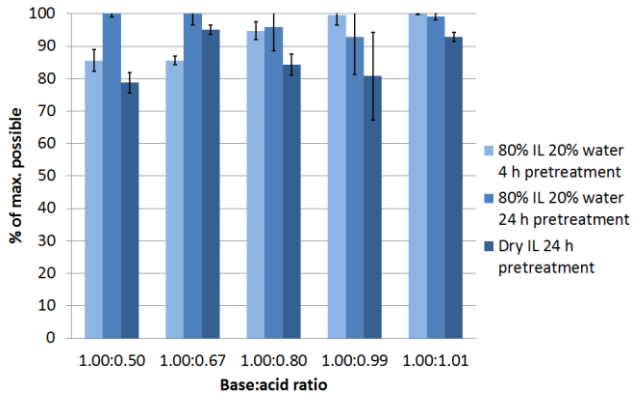


Figure 3 Mass closure for compositional analysis of obtained after deconstruction at 120 ºC.

Effect of ionic liquid solution acidity and treatment time on the pulp composition

Figure 4~~Figure 4~~ shows that hemicellulose removal was more extensive when ionic liquid solution acidity was high. Delignification was also more advanced in highly acidic ionic liquid solutions. Pretreatment efficiency using ionic liquids has been linked to ionic liquid acidity before.[11] Opposed to this, the presence of large amounts of H_2SO_4 led to an increased measured lignin content in the recovered biomass. We ascribe this to the formation of pseudo-lignin from hemicellulose. Pseudo-lignin is insoluble in water and will therefore be measured as acid insoluble lignin. It appears in biomass recovered after deconstruction under relatively severe conditions during dilute acid pretreatment.[12] Saccharification data and visual assessment suggested the pulp was charred when a high excess of sulfuric acid was present.

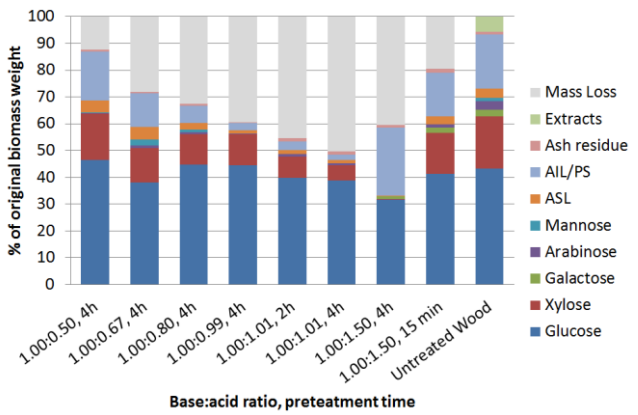


Figure 4 Mass loss and composition of untreated *Miscanthus* and *Miscanthus* pretreated with 80% C_4im:H_2SO_4 20% H_2O mixtures for 4 h or less at 120 ºC. AIL: acid insoluble lignin, ASL, acid soluble lignin, PS: pseudo-lignin.

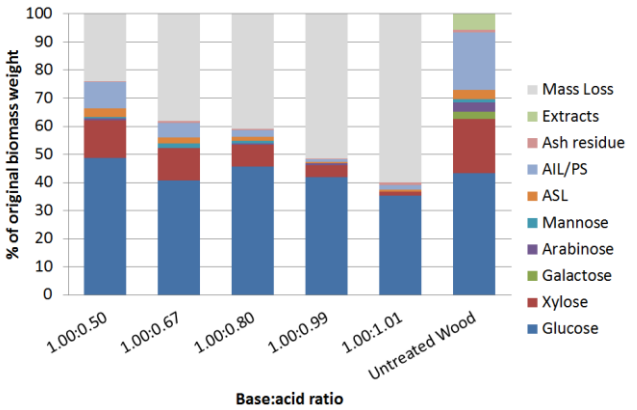


Figure 5 Mass loss and composition of *Miscanthus* pretreated with 80% C_4im:H_2SO_4 20% H_2O mixtures for 24 h at 120 ºC. AIL: acid insoluble lignin, ASL, acid soluble lignin, PS: pseudo-lignin.

The greatest delignification and hemicellulose removal was achieved with aqueous ionic liquid solutions having a base to acid ratio close to 1:1 (C_4im:H_2SO_4 = $[1.00:0.99]_{80\%}$ and = $[1.00:1.01]_{80\%}$) and after a long treatment (Figure 5~~Figure 5~~).

Hemicellulose removal during deconstruction with C_4im:H_2SO_4 = $[1.00:1.01]_{80\%}$ was time dependent. As shown in Table 2 and Figure 6, hemicellulose removal increased from 67% after 2 h to 75% after 4 h and to 94% after a 24 h deconstruction. Lignin removal followed a similar trend, increasing to 91% after 24 h.

Removal of 94% of the hemicelluloses and 91% of the lignin after the 24 h treatment resulted in a pulp with high cellulose content. The pulp's glucan content was 86% (compared to 43% in untreated *Miscanthus giganteus*). However, it should be noted that some of the glucan was solublised during treatment (probably due to the more acidic conditions). Only 82% of the original glucan was present in the pulp, reducing the overall glucan recovery. Other deconstruction conditions allowed recovery of a larger amount of glucan, but resulted in less extensive lignin and hemicellulose removal and hence a less pure pulp.

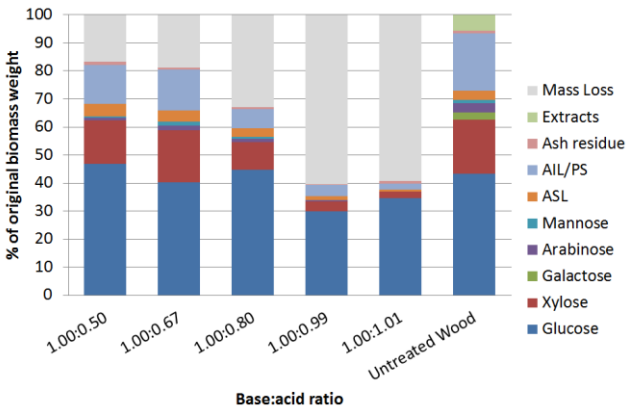


Figure 6 Mass loss and composition of *Miscanthus* pretreated with dry ILs for 24 h at 120 ºC. AIL: acid insoluble lignin, ASL, acid soluble lignin, PS: Pseudo-lignin.

Effect of water content on the pulp composition

The composition of pulps recovered after a 24 h treatment

with dry ILs are shown in Figure 6. Deconstruction with dry C_4im:H_2SO_4 resulted in less delignification and hemicellulose removal when a significant excess of base was present in the ionic liquid solution. The more acidic mixtures resulted in purer pulps, but also reduced glucan recovery.

Saccharification yields

The washed pulps were subjected to enzymatic saccharification and the glucose and hemicellulose yields determined. Figure 7a and 7b show the time courses for these analyses. Although most of the sugar release happened within the first 24 h some sugar release still occured between 72 h and 96 h, suggesting that although 96 h is a good measure for total achievable glucose yield saccharification was not quite complete even after 96 h.

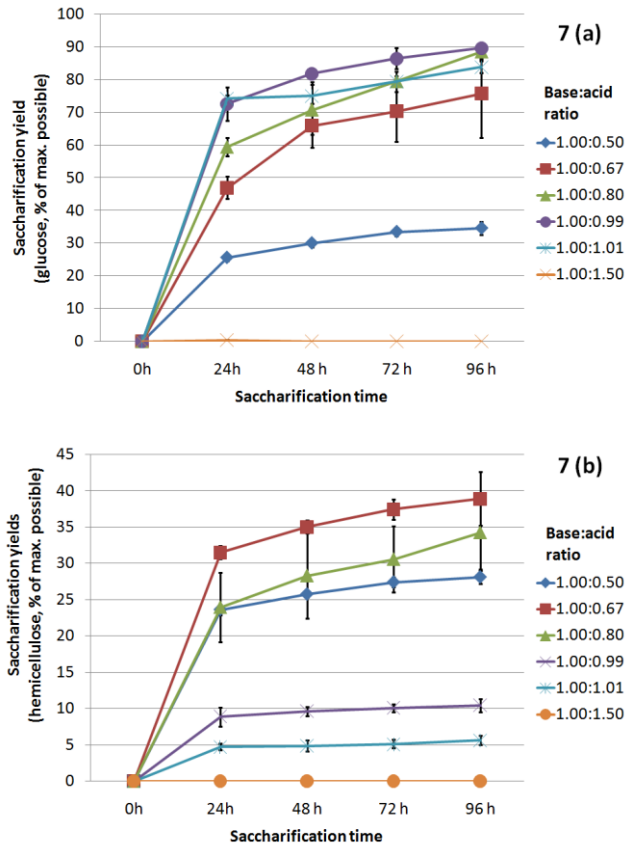


Figure 7 Saccharification yield time course from *Miscanthus giganteus* pulp obtained after deconstruction at 120 ºC for 24 h with 80% IL 20% water mixtures. (a) glucose, (b) hemicellulose (xylose + mannose + galactose). Yields relative to carbohydrate content in the untreated biomass

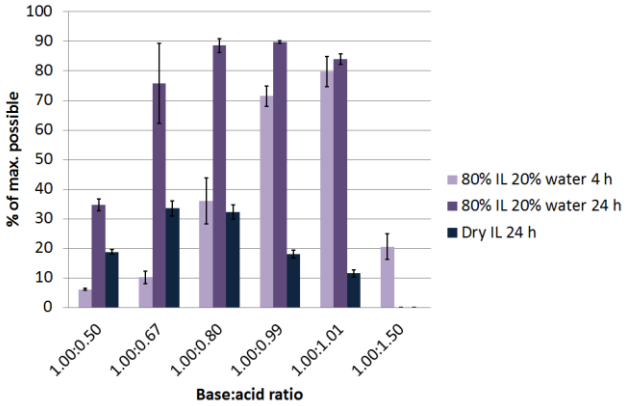


Figure 8 Effect of acidity, water content and treatment time on the glucose yield after 96 h of enzymatic saccharification of *Miscanthus giganteus* pulp pretreated 120 ºC.

Glucose yields

The glucose yields after 96 h of enzymatic saccharification are shown in Figure 8, demonstrating the influence of the IL solution acidity, water content and the deconstruction time. It was observed that the addition of water and longer treatment times resulted in higher glucose yields, while a mid-range acidity was optimal.

The best yields were obtained with IL solutions having mid-range acidity, *e.g.* C_4im:H_2SO_4 = $[1.00:0.80]_{80\%}$ (89%), C_4im:H_2SO_4 = $[1.00:0.99]_{80\%}$ (90%) and C_4im:H_2SO_4 = $[1.00:1.01]_{80\%}$ (84%) after the 24 h treatment.

These yields are comparable with those achieved with $[C_4C_1im][HSO_4]_{80\%}$ and $[C_4C_1im][MeSO_4]_{80\%}$ (up to 90%) in our previous study under similar conditions[66] and demonstrate that the monoalkyl analogue of $[C_4C_1im][HSO_4]$ is equally as effective in deconstructing *Miscanthus*.

Effect of acidity

The use of small amounts of excess acid resulted in high glucose yields after a shorter treatment time. While glucose yields were improved by a 1% excess of acid, they were severely compromised by the presence of 50% excess H_2SO_4. It shows that high saccharification yields were obtained after only 4 h pretreament, while the 1% and 20% excess base solutions required longer treatment to achieve high yields. Figure 9 shows the glucose yields achieved after deconstruction with C_4im:H_2SO_4 = $[1.00:1.01]_{80\%}$ for 2 h (65%), 4 h (80%) and 24 h (84%).

Figure 10 shows that 50% excess acid did not result in good glucose yield even at shorter treatment time. Yields after 15 min and 4 h deconstruction (11% and 20%, respectively) were low and no glucose was enzymatically released after 24 h, showing that excess acid is detrimental to obtaining high saccharificaton yields. Cellulose and glucose are known to be sensitive to degradation under highly acidic conditions. Pretreatment with concentrated sulfuric acid (70%) is typically carried out at temperatures slightly above room temperature, explaining the pronounced degradation effects observed at 120 °C.[13]

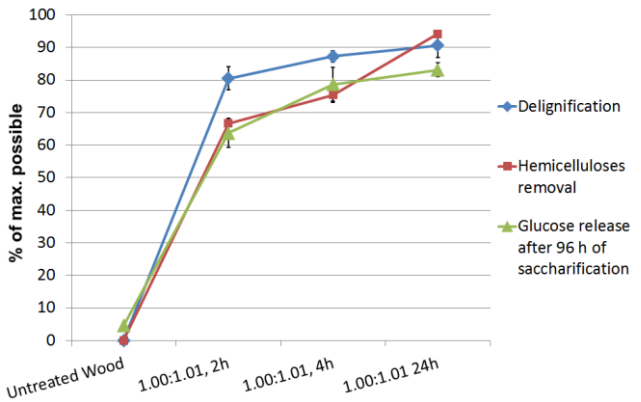


Figure 9 Glucose release after 96 h of enzymatic saccharification and hemicellulose and lignin removal of *Miscanthus* pretreated with 80% C_4im:H_2SO_4 20% H_2O mixtures for 2, 4 and 24 h at 120 ºC

Effect of water

The presence or absence of water had an enormous effect on glucose yields. Although fractionation was advanced in dry C_4im:H_2SO_4 = [1.00:0.99] and C_4im:H_2SO_4 = [1.00:1.01] (Figure 6), the saccharification yields were less than 40% of the theoretical yields. Even if some cellulose degradation is assumed it appears that cellulases were unable to digest the majority of the cellulose in the pulp.

In summary, the highest fermentable glucose recovery was achieved after a 24 h deconstruction with the less acidic aqueous ionic liquid solutions $[1.00:0.80]_{80\%}$ (89%) and C_4im:H_2SO_4 = $[1.00:0.99]_{80\%}$ (90%); whereas, deconstruction with the more acidic solution C_4im:H_2SO_4 = $[1.00:1.01]_{80\%}$ was much faster, achieving promising glucose yields (80%) after only 4 h. These best yields of fermentable glucose in this study (90%) are similar to those found for other efficient deconstruction processes. For example, a study on Organosolv pretreatment of *Miscanthus* achieved 93% over-all fermentable glucose yield (95% glucan recovery and 98% digestibility).[14]

Hemicellulose yields

The trends observed for enzymatic release of hemicellulose sugars (mainly xylose) from the pulp are somewhat different to those for glucose yields (Figure 11). Hemicellulose yields were expected to be lower, as the compositional data show that this fraction had been partially solubilised into the liquid (Figure 4-6).

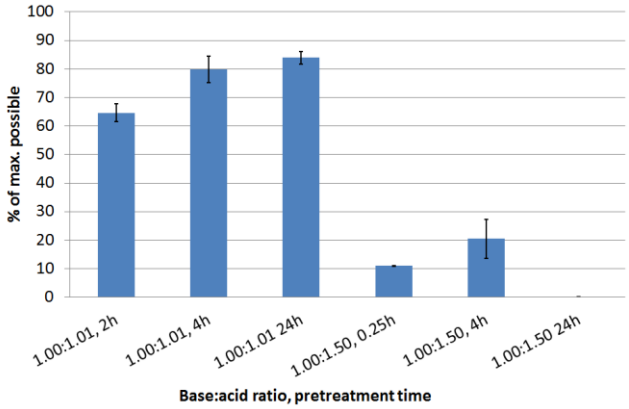


Figure 10 Glucose yields after enzymatic saccharification of pulp deconstructed at 120 ºC with C_4Im:H_2SO_4 = $[1.00:1.01]_{80\%}$ and C_4Im:H_2SO_4 = $[1.00:1.50]_{80\%}$.

The best hemicellulose yields were achieved with ionic liquid solutions containing excess base and water and after a 24 h deconstruction. The use of C_4im:H_2SO_4 = $[1.00:0.67]_{80\%}$ and C_4im:H_2SO_4 = $[1.00:0.80]_{80\%}$ resulted in enzymatic release of 39% and 34% of the hemicellulose sugars. The ionic liquid solution C_4im:H_2SO_4 = $[1.00:0.80]_{80\%}$ also achieved a similar yield (30%) after 4 h of deconstruction. The use of dry ionic liquid resulted in lower hemicellulose saccharificatation yields for the same length of time. As solubilisation of hemicellulose advances over time (Figure 9), the release of hemicellulose from the pulp was higher after 4 h rather than after 24 h.

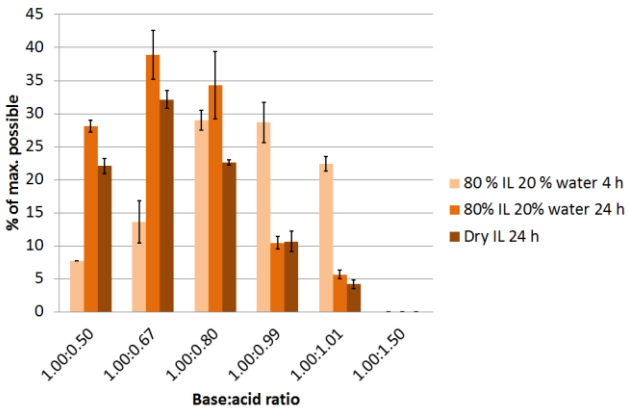


Figure 11 Hemicellulose yields after enzymatic saccharification (96 h) of *Miscanthus giganteus* pretreated with ionic lqiuid solutions at 120 ºC.

These results support the notion that the acidity of $[HSO_4]^-$ containing ionic liquids leads to the depolymerisation and solubilisation of the hemicellulose and hence less hemicellulose is recovered in the pulp. The use of neutral or basic anions, such as methanesulfonate or acetate results in higher hemicellulose yields.[55] For example, xylose yields after deconstruction of ground maple wood with 1-butyl-3-methylimidazolium acetate, $[C_4C_1im][MeCO_2]$, were reported to be as high as 64%,[15] better than the neutral $[C_4C_1im][MeSO_3]$. This is due to the acetate anion being able to control the ionic liquid solution acidity by combining with protons to form acetic acid.[16]

Table 2 Composition of *Miscanthus* pretreated with anhydrous C_4im:H_2SO_4 and 80% IL 20% H_2O mixtures with varying acidity as well as untreated biomass. The treatment conditions were 120 ºC and 15 min - 24 h. The quantity of each components found in the pulp relative to the untreated biomass are given. The mass loss is the matter that dissolved into the ionic liquid.

% recovered relative to the untreated biomass	Time (h)	Glu %	Xyl %	Gal %	Ara %	Man %	Lignin	Ash	Extracts	Mass Loss
Untreated Wood	-	43.2	19.4	2.5	3.2	1.2	23.9	0.9	5.6	0.00
H_2O	24	36.7	3.3	0.0	0.9	0.0	19.1	0.3	0.0	39.7
1.00:0.50/20%	4	46.4	17.2	0.0	0.4	0.1	22.9	0.6	0.0	12.4
1.00:0.50/20%	24	45.7	13.4	0.0	0.8	0.4	12.4	0.3	0.0	24.0
1.00:0.50	24	42.3	15.6	0.0	0.9	0.5	18.3	1.2	0.0	16.7
1.00:0.67/20%	4	38.1	12.8	0.0	0.9	2.3	17.2	0.6	0.0	28.1
1.00:0.67/20%	24	40.8	11.4	0.0	0.2	1.6	7.3	0.5	0.0	38.2
1.00:0.67	24	40.3	18.5	0.0	1.6	15	18.4	0.8	0.0	18.9
1.00:0.80/20%	4	44.8	11.3	0.0	0.8	1.0	9.00	0.6	0.0	32.6
1.00:0.80/20%	24	45.7	7.6	0.0	0.6	0.8	3.7	0.6	0.0	41.0
1.00:0.80	24	44.8	9.8	0.0	1.2	0.77	9.9	0.6	0.0	33.0
1.00:0.99/20%	4	44.5	11.7	0.0	0.1	0.0	3.0	0.1	0.0	39.6
1.00:0.99/20%	24	42.0	4.2	0.0	0.7	0.0	1.4	0.2	0.0	51.5
1.00:0.99	24	29.8	3.6	0.0	0.5	0.0	5.4	0.2	0.0	60.4
1.00:1.01/20%	2	39.9	7.9	0.0	0.9	0.0	4.7	1.4	0.0	45.3
1.00:1.01/20%	4	38.9	5.6	0.0	0.9	0.0	3.0	1.2	0.0	50.3
1.00:1.01/20%	24	35.3	1.5	0.0	0.0	0.0	2.2	1.0	0.0	59.9
1.00:1.01	24	34.6	2.3	0.0	0.0	0.0	2.9	0.9	0.0	59.3
1.00:1.50/20%	0.25	41.2	15.4	1.9	1.2	0.0	19.2	1.6	0.0	12.1
1.00:1.50/20%	4	31.6	0.2	1.2	0.0	0.0	25.5	1.0	0.0	40.5

Lignin recovery

From our previous study we know that a precipitate can be recovered when the lignin containing ionic liquid liquors are diluted with water. The precipitate can be isolated by filtration or centrifugation. We also know from our previous study that the precipitate contains a large proportion of lignin.[66] In addition, the precipitate may also contain water-insoluble hemicellulose oligomers and water insoluble carbohydrate degradation products/pseudo-lignin (particularly after use of more severe treatment conditions). The exact composition of the precipitate remains to be investigated in the future.

For deconstruction with dry C_4im:H_2SO_4 solutions, we observed that increasing the acidity led to increased precipitate yields (Figure 12). In most cases, prolonging the treatment also increased the yield. The absence of added water resulted in slightly reduced yields. The highest precipitate recoveries were obtained with C_4im:H_2SO_4 = [1.00:1.01] solutions, suggesting that more acidic liquors are beneficial for achieving high yields.

The extremely high acidity of the solution containing 50% excess sulfuric acid resulted in poor precipitate yields (Figure 13). This is likely caused by extensive charring of the biomass resulting in decomposition of the lignin into a form where it cannot be redily solubilised into the ionic liquid solution or precipitated from it.

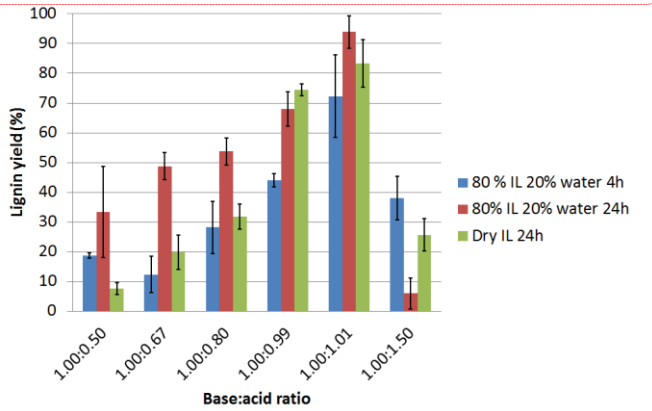


Figure 12 Yield of precipitate relative to lignin content in untreated biomass.

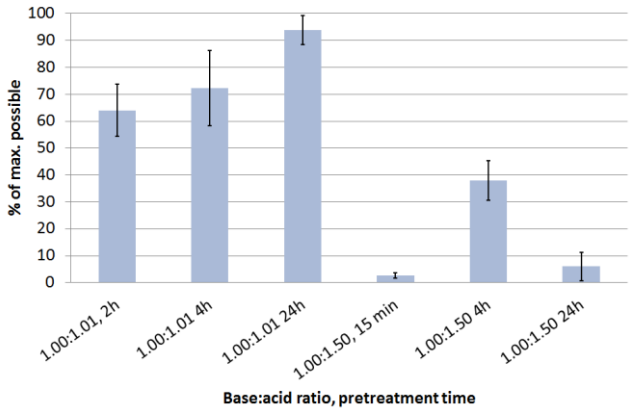


Figure 13 Precipitate yield in acidic IL solutions (relative to the lignin content of the lignocellulose before deconstruction). Treatment was carried out at 120 ºC with C_4im:H_2SO_4 = $[1.00:1.01]_{80\%}$ and $[1.00:1.50]_{80\%}$.

The ratio between precipitate yield and delignification

We also looked at the precipitate yield relative to the delignification. As can be seen in Figure 14, the precipitate yield is typically lower than the delignification, suggesting that some of the dissolved matter is not precipitated from the ionic liquid solution by dilution. The amount of precipitate relative to delignification increased with prolonged pretreatment time and ionic liquid solution acidity (apart from very high acidity). This may be due to alterations in the structure of solubilised lignin such as crosslinking as well as the formation of water-insoluble pseudolignin.

Carbohydrate digestibility

The digestibility tells us how much of the recovered polysaccharide in the pulp is released during enzymatic saccharification. It can be calculated using the glucan and hemicellulose content as determined by compositional analysis and the saccharification yields. It is important to note that the information obtained this way is independent from the total sugar yield, as it is possible to perform deconstructions that only recover a small fraction of the original glucan but in a highly digestible form.

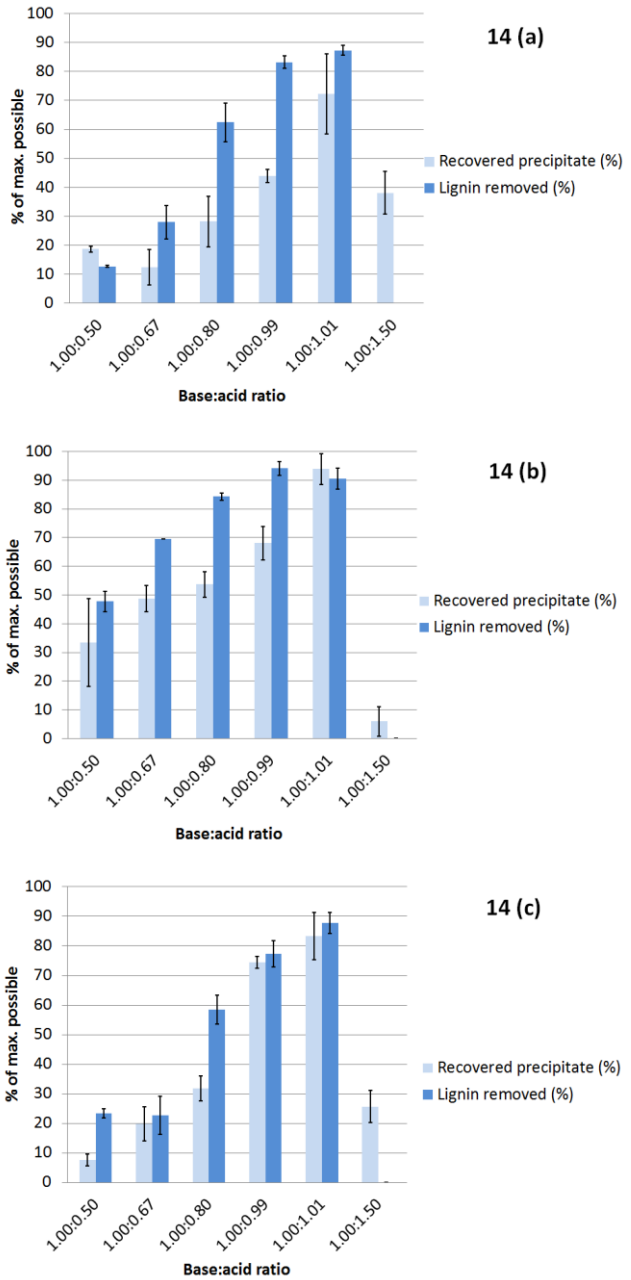


Figure 14 Lignin removal and precipitate yield after deconstruction of *Miscanthus giganteus* with (a) 80% IL 20% water mixtures for 4 h, (b) 80% IL 20% water mixtures for 24 h and (c) with dry ILs for 24 h (relative to the lignin content of the untreated biomass).

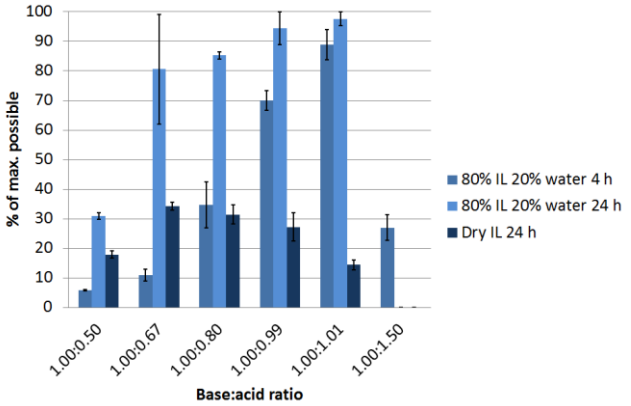


Figure 15 Glucan digestibility of pulps obtained after treatment at 120 ºC and after 96 h of enzymatic saccharification.

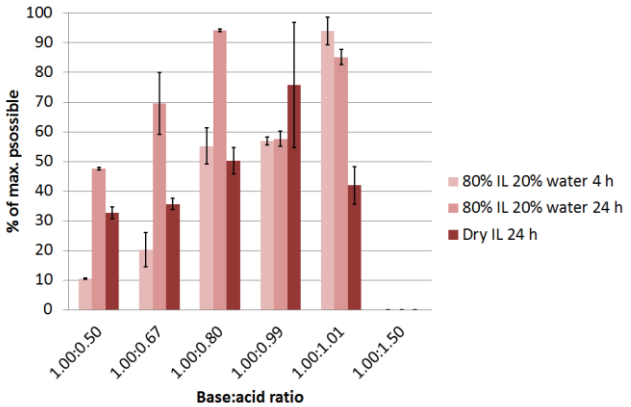


Figure 16 Hemicellulose digestibility of pulps treated with IL solutions at 120 ºC and after 96 h of enzymatic saccharification

Glucan digestibility

The highest glucan digestibilities were obtained after long treatments with mid-range acidic ionic liquid water mixtures (Figure 15). These conditions also resulted in the most extensive hemicellulose and lignin removal (Figure 5). Although the best glucose yields were obtained with $C_4im:H_2SO_4$ = $[1.00:0.99]_{80\%}$ and $C_4im:H_2SO_4$ = $[1.00:0.80]_{80\%}$ (Figure 9**)**, the highest digestibility was measured for pulp obtained after a 24 h treatment with $C_4im:H_2SO_4 = [1.00:1.01]_{80\%}$. Under these conditions, almost complete delignification and hemicellulose removal from the biomass was observed, which in turn resulted in the recovered glucan being saccharified virtually completely within 96 h.

The digestibility of pulp obained after the 4 h treatment was slightly lower (89 %) than after the 24 h treatment (98 %), but the glucose yield was comparable. This is because of the higher glucose recovery after the shorter treatment time (84% and 80%, respectively).

As already mentioned, digestibility was poor for pulp obtained after treatment with dry ionic liquid (Figure 15), even for cases where most of the lignin had been solubilised into the liquor. This suggests that the enzymatic saccharification was being inhibited, perhaps by incomplete removal of the ionic liquid.

Hemicellulose digestibility

In the case of hemicellulose, enzymatic digestibility was also dependent on the treatment conditions. Better hemicellulose digestibility was observed with ionic liquid water mixtures (Figure 16). The digestibility after 4 h of deconstruction increased with IL acidity (apart from highly acidic mixtures), while hemicellulose digestibility was best for pulps treated with less acidic liquors for 24 h, such as $C_4im:H_2SO_4$ = $[1.00:0.80]_{80\%}$, which resulted in the highest hemicellulose digestibility (94%). The use of $C_4im:H_2SO_4$ = $[1.00:1.01]_{80\%}$ for 4 h also led to a hemicellulose digestibility of 94%, but the amount of hemicellulose sugars recovered in the biomass was lower (24% compared to 36% for the biomass pretreated with $C_4im:H_2SO_4 = [1.00:0.80]_{80\%}$).

Experimental

Materials

The reagents 1-butylimidazole (Sigma-Aldrich, 98%), sulfuric acid (VWR BDH Prolabo AnalaR Normapur, 95%) and methanol (VWR BDH Prolabo AnalaR Normapur, 99.8%) were used as received.

The enzymes used for saccharification were cellulase from *Trichoderma reseei* (Sigma-Aldrich) and Novozyme 188 (β-glucosidase from *Aspergillus niger*, Sigma-Aldrich), which can also hydrolyse xylan. The lignocellulosic biomass used in this study was *Miscanthus giganteus* whole stems which were air-dried, ground and sieved (0.18-0.85 mm, -20+80 of US mesh scale) before its use.

Ionic liquids synthesis

A series of 1-butylimidazole/sulfuric acid mixtures were prepared by the dropwise addition of H_2SO_4 ~~(95%)~~ in water (3 ml of water per every 1 ml of H_2SO_4) to a solution of 1-butylimidazole ~~(98.4%)~~ in water (1 ml of water per every 1 ml of 1-butylimidazole). The purity of the starting materials was conisdered when calculating the quantities to be combined. The sulfuric acid for all syntheses originated from a single reagent bottle. Both reagents were dosed using volumetric flasks or graduated borosilicate glass pipettes with B grade or better. The densities used for calculations were 0.945 g/ml for 1-butylimidazole and 1.84 g/ml for the sulfuric acid. The mixtures were stirred at room temperature for several hours.

Once the reaction was complete, the aqueous ionic liquid solutions were decolourised by the addition of charcoal and filtered through neutral alumina. Water was then removed *in vacuo* at 50 ºC, usually for 48 h. ILs were obtained as colourless viscous liquids. Composition and purity of the ILs were confirmed by ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy; these data are given in the electronic supplementary information.

NMR spectra were recorded on a Bruker 400 MHz instrument with chemical shifts given in ppm and coupling constants in Hertz. LSIMS spectra were recorded on a Micromass AutoSpec Premier spectrometer using 3-

nitrobenzyl alcohol as the matrix.

Biomass deconstruction

Moisture determination in biomass and ionic liquid

The oven-dry weight (ODW) of lignocellulose biomass was determined according to the procedure described in the LAP "Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples" (NREL/TP-510-42621). In brief, air-dried biomass (100-200 mg) was placed on aluminum foil of known weight and dried at 105 °C overnight. The samples were transferred into a desiccator with activated silica for cooling and the weight determined using a Sartorius CP423S balance. The moisture content was calculated according to Eq. 1:

$$Moisture(\%) = \frac{m_{airdried} - m_{ovendried}}{m_{ovendried}} \cdot 100\% \quad \text{Eq. 1}$$

The ionic liquids used in this study absorb substantial amounts of water from the air and therefore require drying prior to use to control their water content. The water content of ionic liquids was measured with a Mettler Toledo DL32 coulometric Karl-Fischer titrator or a TitroLine KFtrace (Schott Instruments). After water content determination, they were immediately used for deconstruction.

Deconstruction of *Miscanthus giganteus*

Deconstruction was carried out by placing 1.040-1.073 g air-dried biomass (1.000 g oven-dried weight) into Pyrex culture tubes with teflon-lined screw caps. 10 ml of IL solution or IL water mixture (80: 20 vol%) was added and the samples incubated at 120 ºC for 15 min up to 24 h without stirring. All experiments were run in duplicate.

After incubation, samples were allowed to cool to room temperature, mixed with 10 ml methanol and left to equilibrate for 4 h. The suspension was filtered and the supernatant set aside for lignin yield determination. The solids (pulps) were purged with methanol and incubated with 10 ml of fresh methanol for 24 h. The solids were filtered again, washed with more methanol and air-dried overnight. An additional washing step was introduced in the last set of experiments. The air-dried weight of the pulps was recorded and their moisture content determined according to Eq. 1.

Enzymatic saccharification

The air-dried pulps were subjected to enzymatic saccharification following the LAP procedure "Enzymatic Saccharification of Lignocellulosic Biomass" (NREL/TP-510-42629). 150 mg pulp was mixed with 9.85 ml of a solution containing 5.00 ml of citrate buffer, 4.66 ml of deionised water, 40 µl of tetracycline, 30 µl of cycloheximide, 59 µl of cellulase and 59 µl of β-glucosidase. Samples were incubated at 50 ºC for 96 h with shaking rotation (250 rpm).

In order to study sugars release with time, 700 µl samples containing both liquid and solid were collected every 24 h. The solids were removed by centrifugation and the sugar content analyzed using a Jasco HPLC system equipped with an Aminex HPX-87H column (Biorad). The mobile phase was 10 mM sulfuric acid, the column oven temperature was set to 55 ºC, the flow rate was 0.6 ml·min^{-1} and the acquisition time 25 min.

Xylose, mannose and galactose elute at the same time under these conditions. As xylose is the main contribution in *Miscanthus* the anhydro correction and the extinction coefficient for xylose was applied for this peak.

Compositional analysis

Compositional analysis of untreated *Miscanthus giganteus* and the pulps was carried out following four LAP procedures, such as "Preparation of samples for compositional analysis" (NREL/TP-510-42620) and "Determination of Structural Carbohydrates and Lignin in Biomass" (NREL/TP-510-42618).

Total solids was determined using "Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples" (NREL/TP-510-42621), method B, "convention oven drying". The extractives in untreated *Miscanthus gigantheus* was quantified according to the LAP "Determination of extractives in biomass" NREL/TP-510-42619. 3.000 g of air dried *Miscanthus gigantheus* were extracted two times with ASE 300 accelerated solvent extractor (Dionex), the first time with deionised water and the second time with ethanol (95%).

Carbohydrates were analysed on an Agilent 1200 system equipped with an Aminex HPX-87P column, a deashing column and a Carbo-P guard column (all Biorad). The mobile phase was deionised water, the column temperature was set to 80 ºC, the flow rate was set to 0.6 ml·min^{-1} and the acquisition time was 35 min. For each sugar, a calibration curve was created by linear regression. These curves were used for determining the concentration of each component present in mg·ml^{-1}.

The acid soluble lignin content (ASL) was measured using the UV absorbance at 320 nm in a suitable dilution. ε = Absorptivity of acid soluble lignin at the specified wavelength, in this case 25 L/g cm.

Once the compositional analysis of untreated *Miscanthus giganteus* was carried out, the extractives content (in %) was normalized together with the percents of each component of the extracted biomass, as obtained with the compositional analysis.

Lignin recovery

The supernatant obtained after deconstruction was dried under vacuum at 40°C using a Carousel 12 parallel synthesizer (Radleys, UK) to remove methanol. 30 ml of water were added to the dried liquor to precipitate the lignin fraction. The precipitate was washed 2 times with 25 ml distilled water, air-dried overnight and dried under vacuum at 50 °C to obtain the oven-dried weight. The precipitate yield was determined relative to the lignin content of the original biomass according to Eq. 2.

$$Precipitate\ yield\ (\%) = \frac{m_{precipitate}}{m_{lignin\ (\text{comp. analysis})}} \cdot 100\% \quad \text{Eq. 2}$$

Biomass digestibility

Once saccharification yields and the composition of the pretreated samples was determined, equation 3 was employed in order to calculate digestibility after enzymatic saccharification.

$$\%\ Digestibility = \frac{saccharification\ yield}{glucan\ in\ biomass}\ x\ 100\% \qquad \text{Eq. 3}$$

Conclusions

Protic IL solutions prepared by combining 1-butylimidazole and sulfuric acid were sucessfully applied in the fractionation of *Miscanthus giganteus* into a cellulose rich pulp and a lignin-rich precipitate at 120 °C.

We observed that the ionic liquid solution acidity and the deconstruction time substantially influenced the fractionation and the glucan recovery as well as the saccharification yield. Long deconstructions were more effective in removing lignin and hemicellulose but could also result in loss of glucan into the liquor.

A slight excess of acid led to higher cellulose content in the pulp and accelerated pretreatment, whereas reduced acidity resulted in a higher hemicellulose recovery with the solids. A substantial excess of acid led to poor fractionation and degradation of carbohydrates and likely also lignin at 120 °C, suggesting that liquors with a slight excess of acid or base may be best suited for this application.

It was observed that the saccharification yield and glucan digestibility were sensitive to the presence of added water. They were unsually low in the absence of water, suggesting that the use of dry ionic liquid treatment negatively affects of the access of saccharifcation enzymes to the polysaccharides.

It was found that digestibility of cellulose pretreated with IL water mixtures was linked to lignin and hemicellulose removal while it was not for dry IL. In the case of the hemicellulose sugars, we observed that the enzymatic digestibility was linked to the delignification and hemicelluloses removal (the latter limiting the overall xylose yield that can be achieved).

The possibility of achieving high saccharification yields with inexpensive, protic ionic liquids within a relatively short time is of great interest to industrial applicability of Ionosolv pretreatment. The economic attractiveness of the Ionosolv process will be further enhanced if repeated reuse (recycling) of the ionic liquid can be demonstrated in the future.

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Notes and references

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