Understanding the Interactions of Cellulose with Ionic Liquids: A Molecular Dynamics Study

ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · MARCH 2010

Impact Factor: 3.3 · DOI: 10.1021/jp9117437 · Source: PubMed

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Impact of mixed feedstocks and feedstock densification on ionic liquid pretreatment efficiency

Biofuels (2013) 4(1), 63–72

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Background: Lignocellulosic biorefineries must be able to efficiently process the regional feedstocks that are available at cost-competitive prices year round. These feedstocks typically have low energy densities and vary significantly in composition. One potential solution to these issues is blending and/or densifying the feedstocks in order to create a uniform feedstock. **Results & discussion:** We have mixed four feedstocks – switchgrass, lodgepole pine, corn stover and eucalyptus – in flour and pellet form, and processed them using the ionic liquid 1-ethyl-3-methylimidazolium acetate. Sugar yields from both the mixed flour and pelletized feedstocks reach 90% within 24 h of saccharification. **Conclusion:** Mixed feedstocks, in either flour or pellet form, are efficiently processed using this pretreatment process, and demonstrate that this approach has significant potential.

In order to accomplish large-scale utilization of biomass feedstocks to produce biofuels, a consistent and stable supply of sustainable feedstocks from a variety of sources will be required. Complicating this further is that feedstock diversity varies markedly from region-to-region in the USA, and each feedstock within a given region varies from year-to-year based on weather conditions, handling, storage and crop variety [1]. In order to maintain productivity and profitability, a biorefinery must be able to efficiently convert those feedstocks that are available at required levels and at affordable prices. These feedstocks will be diverse and will change as a function of time and price, and will most likely be available in a mixed input stream to the biorefinery. The biorefinery must be able to process these **mixed biomass feedstocks** with minimal negative impact in terms of overall performance, sugar production and fuel titers. Unfortunately, the majority of the scientific literature available on biomass conversion has typically focused on the conversion of one feedstock, and little attention has been paid to the efficiency of converting mixtures of feedstocks into fermentable sugars and fuels. Moreover, most of the pretreatment technologies studied are highly effective in handling a specific range of feedstocks, but there are very few conversion technologies with a demonstrated ability to handle a wide range of feedstocks with minimal negative impact on efficiency and sugar yields. **Ionic liquid (IL) pretreatment** is unique in that it is capable of efficiently handling softwoods [2–4], hardwoods [4–6], herbaceous materials [7–9] and agricultural residues [10–13] as single feedstocks. However, little attention has been given to the performance of ILs in the conversion of mixed feedstocks.

One approach to avoid a significant drop in biomass conversion efficiency is to develop a formulation of mixed feedstocks in order to produce a more consistent material. Formulation combines various preprocessed biomass resources and/or additives to produce an on-spec feedstock that is capable of being traded and used as a commodity. The resulting feedstock will provide

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10.4155/BFS.12.82 ISSN 1759-7269

Key terms

Mixed biomass feedstocks: Substrate that is generated by taking feedstocks of different types and mechanically mixing them together in order to help alleviate annual or seasonal fluctuations in price and/or availability.

Ionic liquid pretreatment: Thermochemical process where ionic liquids are mixed with biomass and heated for a set time interval, followed by addition of an antisolvent, typically water or ethanol, to recover the biomass.

Biomass blending: Process in which feedstocks of different types are mixed together to generate a substrate that meets a targeted range of compositional specifications to make it suitable for downstream conversion.

Biomass densification: Mechanical process by which native feedstocks are compressed to increase the energy density of the material and enhance the transportation efficiency of the material from the farm to the gate of the biorefinery.

consistency and lower costs to bioenergy industries because they can design their processes around a single feedstock. The overarching goal of biomass formulation is to facilitate the use of consistent feedstocks composed of different and variable sources of biomass. Feedstock formulation is not a new concept in many market sectors. For example, different grades of coal are blended to reduce sulfur and nitrogen contents for power generation [14], animal feeds are blended to balance nutrient content [15] and forage crops are amended with lactic acid bacteria in silage piles to improve digestibility and enhance aerobic stability [16]. **Biomass blending** feedstocks refers to the combination of multiple sources of the same biomass resource to average out compositional and moisture variations, whereas aggregation refers to the combination of different raw or preprocessed biomass resources to produce a single, consistent feedstock with desirable properties. Examples include mixing blended corn stover with blended switchgrass; mixing blended wheat straw with blended softwood residuals; and mixing blended *Miscanthus* with blended rice hulls. This strategy will allow desirable characteristics of many types of feedstocks to be combined to achieve a better feedstock than any of the feedstocks alone.

In addition to these formulation approaches, another consideration that must be taken into account is the energy density of the feedstock itself. Energy density plays a significant role in the overall energy and cost balance of the biofuel production process. Simply put, a biomass feedstock with low energy density is less energy efficient to convert into a biofuel than one with a higher energy density due to the relatively high energy required to transport, store and distribute the feedstock from the field to the biorefinery gate [17,18]. **Biomass densification** typically involves exposing the biomass to elevated pressures and temperatures to remove excess water and compress the biomass. This process acts as a mild thermochemical pretreatment and can also impact the composition and structure of the biomass. There are several different densification forms, including bales, briquettes, pellets, cubes and pucks. These have all been demonstrated as capable of significantly increasing the energy density of biomass, primarily focused on the production of homogeneous feedstocks, such as corn stover and switchgrass [19,20]. There are a few reports in the scientific literature indicating that for certain combinations of densified feedstock and pretreatment, for example dilute acid and alkaline pretreatment of pelleted switchgrass, there is no adverse impact of densification [21].

All of the considerations stated above underscore the need to develop and optimize a biomass conversion technology capable of handling a wide range of feedstocks that is available, affordable and consistent. Our hypothesis is that IL pretreatment with 1-ethyl-3-methylimidazolium acetate ($[C_2mim][OAc]$) is capable of efficiently handling mixed feedstocks that have been milled and densified into pellets, and can generate high yields of fermentable sugars regardless of upstream processing. To test that hypothesis, we have mixed four biomass feedstocks that represent the general classes available: lodgepole pine (*Pinus contorta*), eucalyptus (*Eucalyptus cinerea*), corn stover (*Zea mays*) and switchgrass (*Panicum virgatum*). These feedstocks were mixed together as flour and as pellets to determine the impact of mixing and densification on conversion efficiency.

Experimental

Materials

The four feedstocks included in this study were corn stover, switchgrass, lodgepole pine and eucalyptus. The corn stover was grown near Emmitsburgh (IA, USA) and was harvested in September 2010. Switchgrass was grown near Guymon (OK, USA) and was harvested in October 2010. Both of these materials were delivered to Idaho National Laboratory (INL; ID, USA) in the form of 3 × 4 × 8 ft bales and have been stored since delivery under tarps. Lodgepole pine trees ranging from 3 to 12 inches in diameter at the trunk were harvested in May 2011 in Island Park (ID, USA) by Wilcox Logging, located in Rigby (ID, USA). The trees were shredded using a tub grinder by Wilcox Logging and were not debarked or delimbed prior to shredding. The shredded pine was stored at the INL in piles on an asphalt pad. The eucalyptus was harvested in Davis (CA, USA) with tree sizes ranging up to 18 inches in diameter. The trees were delimbed for shipment, but not debarked. These trees were also shredded as described above for the lodgepole pine. The shredded eucalyptus was also stored at the INL on the asphalt pad in the same manner as the lodgepole pine.

Grinding

The corn stover and switchgrass bales were ground using a Vermeer BG480 grinder (Vermeer, IA, USA) designed for processing up to 4 × 4 ft bales. A 1-inch screen was used for these grinds. Lodgepole and eucalyptus were ground using a Vermeer HG200 modified to use a chipping style drum with a three-quarter inch hexagon shape screen used to prevent oversized material from continuing further in the process. The woody materials were conveyed to a Baker-Rullman SD75-22 Dryer System (Baker-Rullman, WI, USA) with a 10 mmBtu/h CNFGD Kinedizer LE Burner (Maxon Corporation, IN, USA) equipped with a prepiped and prewired propane gas train and stainless steel burner internals. Residence time in the drier was controlled by the outlet temperature and the airflow rate in the drier. Each feedstock was then ground in a model E-4424-TF hammer mill made by Bliss Industries (OK, USA). A 3/16-inch screen was used for these grinds.

Mixing & pelleting

After the grinding and drying steps, the four ground feedstocks were conveyed into a metering bin for mixing. Equal quantities of each feedstock (dry weight basis) were layered into the metering bin and conveyers were used to cycle the material from the bin outlet back to the inlet for mixing. After mixing, steam conditioning was used to increase the temperature of the blended material prior to entering the pellet mill. The mixed feedstock material was pelleted with a softwood die using a B200B-1209 Bliss Pioneer Pellet Mill manufactured by Bliss Industries. This die had a compression ratio of 8:1. The diameter pellets produced from this die were one-quarter inch. The length of the pellets was set to be less than 2.5 inches. Samples were pulled throughout the grinding, drying, mixing and pelleting process to assess moisture content by drying for 24 h at 103°C.

IL pretreatment

[C_2mim][OAc] was purchased from BASF (NJ, USA) and used as the IL for all pretreatments in this study. The mixed flour and pelleted samples were pretreated with [C_2mim][OAc] at 160°C for 3 h in an automated 500-ml Globe Chemical Reactor system (Syrris, Inc., MA, USA) in an air atmosphere. 20 g of biomass (dry basis) were mixed with 180 g of [C_2mim][OAc] to give a 10 wt% biomass loading. Pretreatment runs were carried out with constant stirring at 150 rpm by a 80 mm diameter polytetrafluoroethylene anchor-type impeller, powered by a Heidolph RZR 2052 mechanical stirrer (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). Duplicate runs were performed for each IL pretreatment of mixed flour and pellets. After pretreatment, 400-ml hot water was added to the samples as antisolvent for cellulose regeneration and for recovering the majority of the solubilized biomass, although lignin and hemicellulose are expected to remain solubilized after antisolvent addition. The mixture of IL, water and recovered biomass was centrifuged at 3220 relative centrifugal force for 10 min to separate the solids and liquid phases. The recovered biomass was washed four times with 1000 ml of hot water to remove any excess [C_2mim][OAc]. An aliquot of recovered solid was lyophilized in a FreeZone® Freeze Dry System (Labconco, MO, USA) and used for composition and x-ray diffraction (XRD) analysis.

Compositional analysis

Acid-insoluble lignin and structural carbohydrates (including glucan, xylan, arabinan, galactan and mannan) of mixed flour and pellet samples, before and after pretreatment, were determined according to the two-step acid hydrolysis procedure of the National Renewable Energy Laboratory (NREL; CO, USA). The other components expected to be present in the biomass were not determined. Acid hydrolysis samples were diluted 100-fold and analyzed by high-performance anion-exchange chromatography (HPAEC) on an ICS-3000 system (Dionex, CA, USA) equipped with an eluent generator, an electrochemical detector and a 4 × 250-mm Dionex CarboPac™ PA20 analytical column. Pretreatment liquid was diluted 100-fold and measured directly by HPAEC for monomeric sugars. Furthermore, for oligomers determination, an aliquot of pretreatment liquid was mixed with an equal volume aliquot of 72% H_2SO_4, incubated at 30°C for 1 h, diluted to 4% sulfuric acid concentration with deioinized (DI) water and autoclaved at 121°C for 1 h (posthydrolysis) according to previously published NREL laboratory analytical protocol, 'Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples'. Differences between the amount of sugars following posthydrolysis and the monomer content before posthydrolysis were defined as the oligomeric sugar content.

X-ray powder diffraction measurements

XRD data were collected with an Empyrean x-ray diffractometer (PANalytical, MA, USA) equipped with a PANalytical PIXcel^{3D} detector and operated at 45 kV and 40 kA using Cu Kα radiation (λ = 1.5418 Å). The patterns were collected in the 2θ range of 5–55°, the step size was 0.026° with an exposure time of 600 s. The pelleted samples were ground by hand to eliminate any potential artifacts due to the cylindrical form factor and reduce the background. A reflection-transmission spinner was used as a sample holder and the spinning rate was set at 8 rpm throughout the experiment. The crystallinity index (CrI) was determined from the ratio of the crystalline peak area to the total area using the software package HighScore Plus®.

Enzymatic hydrolysis

Enzymatic saccharification runs of pretreated and untreated biomass samples were run in duplicates and adhered to the NREL laboratory analytical protocol 9 'Enzymatic Saccharification of Lignocellulosic Biomass' at NREL standard conditions (50°C, 0.05 M citrate buffer and pH 4.8). The citrate buffer (final molarity 50 mM), sodium azide (antimicrobial; final concentration of 0.01 g/l), enzymes and DI water were mixed with the recovered solids after pretreatment to achieve a final solids loading of around 2% (equivalent to 1% [w/w] glucan concentration). Cellulase (Cellic® CTec2; Batch# VCN10001, protein content 188 mg/ml) and hemicellulase (Cellic® HTec2; Batch# VHN00001, protein content 27 mg/ml) enzyme mixtures, gifts from Novozymes N.A. (NC, USA), were used at enzyme loadings of 20 mg CTec2 protein/g glucan supplemented with HTec2 at loading of 0.26 mg enzyme protein/g glucan unless otherwise specified. The supernatant collected during 72 h of hydrolysis was analyzed with HPAEC for the monosaccharide as described in the compositional analysis section above. Enzymatic digestibility was defined as the glucose yield based on the maximum potential glucose from glucan in biomass. After 72 h of hydrolysis, the remaining solids were collected by centrifugation and washed with an excess volume of DI water to remove residual sugars. The solids were then lyophilized and analyzed for acid-insoluble lignin, glucan and xylan compositions.

Results & discussion

▪ Moisture content of mixed flour & pellet feedstocks

The moisture content of the four feedstocks as a function of location within the process is shown in **Figure 1**. The starting moisture contents for the corn stover bales, switchgrass bales and the shredded lodgepole pine and eucalyptus were 14.6, 7.5, 37 and 40%, respectively. Per manufacturer's instructions, the optimal moisture content for pelleting is typically between 10 and 14% depending upon the feedstock. Based upon this, the woody materials were dried prior to further processing. The moisture content of these materials was reduced to 15.8% after drying. The moisture content of the herbaceous feedstock was measured after the first stage grinder (Vermeer BG480) and before the dryer (labeled 'before dryer' on **Figure 1**) to be 11.3 and 8.1%, respectively, for the corn stover and switchgrass. Further drying of the woody materials also occurred during the hammer mill grind, but had little impact on the moisture content of the herbaceous material. The four feedstocks were cycled through the metering bin four times to achieve complete mixing (assessed visually) and the resulting mix had an overall moisture content of 11.1% prior to pelleting.

▪ Chemical composition & solid recovery before & after pretreatment with [C_2mim][OAc]

Previous studies have established optimal IL pretreatment conditions for corn stover [10], switchgrass [7] and eucalyptus [22]. Although it is generally believed that softwoods are highly recalcitrant and require a severe pretreatment stage in order to achieve efficient enzymatic conversion of polysaccharides into fermentable sugars, IL pretreatment with [C_2mim][OAc] showed similar high efficiency improving digestibility of pine using conditions similar to those applied to other biomass feedstocks [2]. This indicates that pretreatment with [C_2mim][OAc] under certain conditions is effective on multiple feedstocks. This has been further demonstrated in a recent study, where a single pretreatment condition using [C_2mim][OAc] (160°C, 3 h) was applied to three biomass types with high sugar yields produced, albeit it used lower biomass loadings (3 wt%) than the current study [23]. The feedstock agnostic feature of IL pretreatment using [C_2mim][OAc] makes it commercially favorable as an IL pretreatment-based biorefinery, and enables a broad operation window for mixed feedstocks.

Images of the IL pretreatment process of the mixed flour and pellet feedstocks are presented in **Figure 2**. It is worth noting that although the pellets are denser than the flour, the pellets were observed to quickly swell and partially solubilize during pretreatment with [C_2mim][OAc] in a manner very similar to that observed for the flour. The feedstock compositions before and after pretreatment are listed in **Table 1**, and the results after pretreatment for both the flour and pellets were similar in glucan, xylan, arabinan, galactan, mannan and acid-insoluble lignin content. It is also noted that there was 2.8 and 2.3% mannan in the mixed flour and mixed pellets, respectively, and is attributed to the presence of lodgepole pine. Compared with the starting materials, the IL-pretreated mixed flour had decreased xylan contents (9.2%) and enriched glucan content (48.9%), which is similar to IL-pretreated mixed pellets (8.9% of xylan and 49.7% of glucan). It is also noted the there was a decrease in other minor polysaccharides; that is, arabinan, galactan and mannan, in the [C_2mim][OAc] pretreated solids, due to the simultaneous dissolution of hemicellulose components [24]. Although it is well known that [C_2mim][OAc] is capable of solubilizing both cellulose and lignin, the lignin content in the pretreated mixed flour and pellets is similar to that of the untreated materials [23,24]. We attribute this to the simultaneous removal of glucan, xylan and other minor polysaccharides and lignin, resulting in similar lignin contents in the resulting solids after pretreatment with [C_2mim][OAc] of the mixed feedstocks. These results are slightly different than those previously published at 3 wt% biomass loading, and we attribute these differences to the limits of biomass solubilization due to the high biomass loading used in the current study [23,24].

Table 1 also shows that, after [C_2mim][OAc] pretreatment, approximately 64.9% of the starting mixed flour biomass was recovered as pretreated solids. A similar solid recovery of 63.1% was obtained after pretreatment of the mixed pellets. The mass loss is attributed to the solubilization of components such as lignin, xylan and other soluble extractives. The loss of the initial glucan fraction was approximately 10% in both mixed flour and pellets, but the removal of xylan was significantly higher (66.1 and 64.7% of for mixed flour and pellets, respectively). The removal of minor polysaccharides (i.e., arabinan, galactan and mannan) was similar to xylan removal, likely due to the associated hemicellullose dissolution. The lignin removal observed in this study (34.9 and 35.7% for mixed flour and pellets, respectively) was lower than reported elsewhere for corn stover (57.2%) and switchgrass (70.6%) [23,24]. We hypothesize that this difference arises from the presence of more recalcitrant feedstocks, such as pine, and the overall lignin removal of mixed feedstocks is an average of all four types of biomass present [23]. The solid loading and cellulose regeneration method may also contribute to the lower lignin removal observed in this study. Compositional results clearly demonstrate

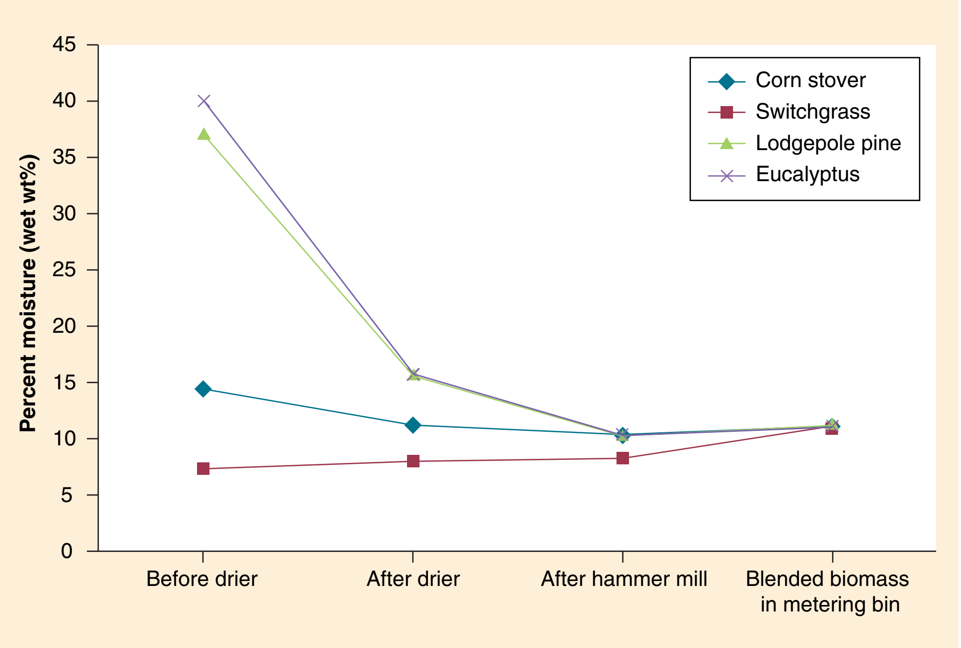

Figure 1. Moisture content of feedstocks studied as a function of process.

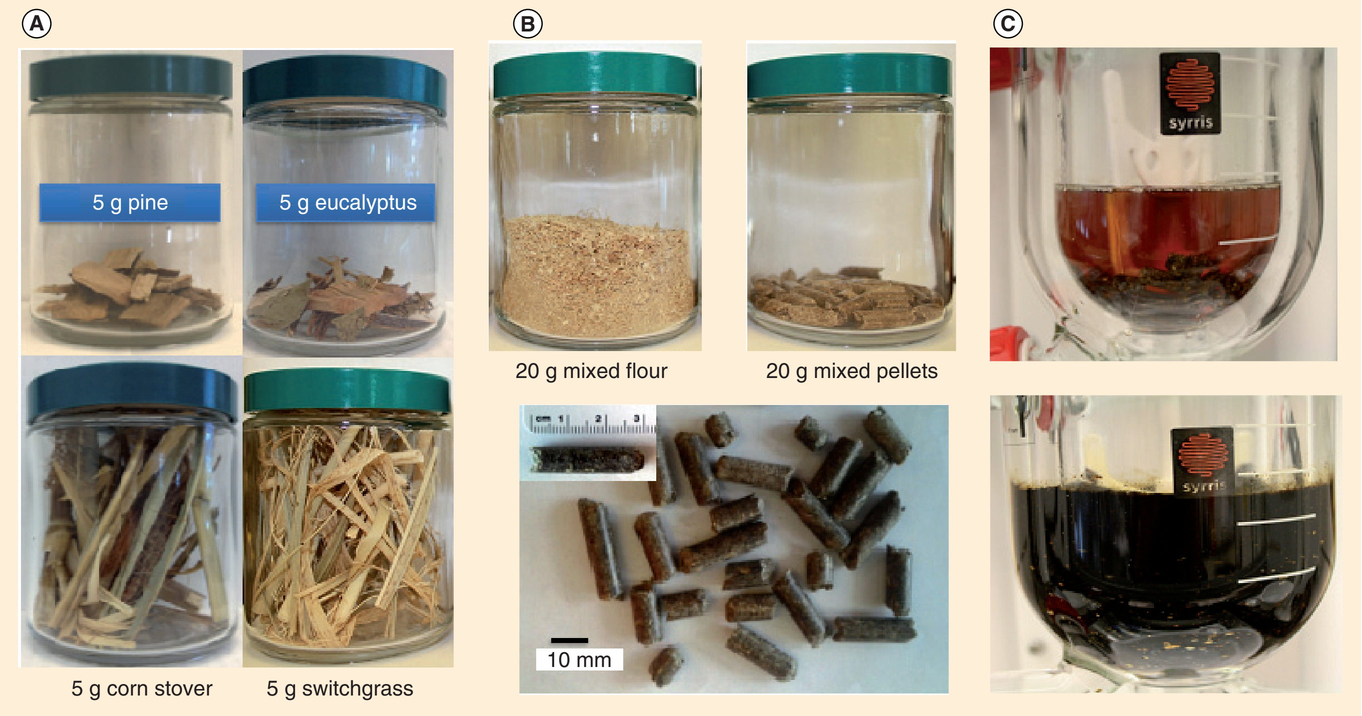

Figure 2. Pretreatment of mixed flour and pellets by 1-ethyl-3-methylimidazolium acetate. Images depicting **(A)** initial single feedstocks, **(B)** the different format of the mixed flour and pellets and **(C)** the process of pretreatment on 1-ethyl-3-methylimidazolium acetate on the mixed pellets where the initial material is observed to undergo expansion and solubilization at 160°C.

Table 1. Chemical composition of dominant sugars and lignin in the feedstocks studied, solid recovery and component removal of mixed biomass feedstocks before and after ionic liquid pretreatment†.

Treatment	Solid recovery‡ (%)	Glucan (%)	Xylan (%)	Arabinan (%)	Galactan (%)	Mannan (%)	Lignin (%)
Mixed flour							
Untreated	100.0	35.6 ± 1.6	17.7 ± 0.8	2.0 ± 0.1	2.0 ± 0.1	2.8 ± 0.1	24.7 ± 1.3
IL pretreated	64.9 ± 1.8	48.9 ± 0.6	9.2 ± 1.1	1.2 ± 0.2	1.0 ± 0.1	1.4 ± 0.4	24.7 ± 0.6
% reduced	35.1	10.9 ± 0.4	66.1 ± 0.7	61.7 ± 0.1	67.7 ± 0.1	67.8 ± 0.3	34.9 ± 1.6
Mixed pellets							
Untreated	100.0	34.7 ± 0.2	15.9 ± 0.2	1.9 ± 0.3	1.9 ± 0.6	2.3 ± 0.4	23.9 ± 0.1
IL pretreated	63.1 ± 4.7	49.7 ± 1.6	8.9 ± 1.3	1.0 ± 0.2	0.9 ± 0.4	1.5 ± 0.1	23.7 ± 0.3
% reduced	36.9	9.5 ± 1.0	64.7 ± 0.8	66.8 ± 0.1	70.1 ± 0.2	57.7 ± 0.0	35.7 ± 0.2

†Reduction (%) represents the percentage removal of each component on the mass basis of its original content in untreated material.
‡Values based on the weight of untreated material.
IL: Ionic liquid.

Key term

Biomass pretreatment: Thermochemical process by which the surface area of biomass is increased and/or biomass composition is altered in order to generate a substrate that is easier to hydrolyze using enzymes as compared with the starting material.

that the IL pretreatment was similarly effective on both mixed flour and pellets.

X-ray diffraction

We performed XRD measurements to investigate the impact of IL pretreatment on the cyrstallinity present in both the mixed flour and pellet feedstocks (Figure 3). The degree of crystallinity in lignocellulose, primarily attributed to the microcrystalline cellulose present in the plant cell walls, has been well established as one of the main factors in determining the efficiency of enzymatic hydrolysis [25–29]. Native cellulose consists of crystalline domains and amorphous regions. Four different polymorphs of cellulose (cellulose I–IV) are known [30]. Cellulose I is the natural polymorph present in the plant cell wall, whereas the other three structures are obtained by regeneration or exposure to various processes [30]. The XRD patterns of the untreated mixed feedstocks display typical semi-amorphous structure with the crystalline component being cellulose I (Figure 3). The major diffraction peaks at 22.2° and 22.3° 2θ for the mixed flour and mixed pellets, respectively, correspond to the separation between the hydrogen-bonded sheets in cellulose I. A broad peak at approximately 15.1–16.5° is also observed and represents a combination of the 101 and 10$\bar{1}$ reflections. The CrI for the untreated mixed flour and pellets is 0.37 and 0.33, respectively, indicating that the microcrystalline cellulose is not significantly altered as a result of pelletization. After treatment with [C_2mim][OAc], the crystalline structure is significantly altered and the samples essentially display amorphous structures with limited long-range order (Figure 3). The calculated residual CrI for the IL-treated mixed flour and pellets are 0.03 and 0.05, respectively.

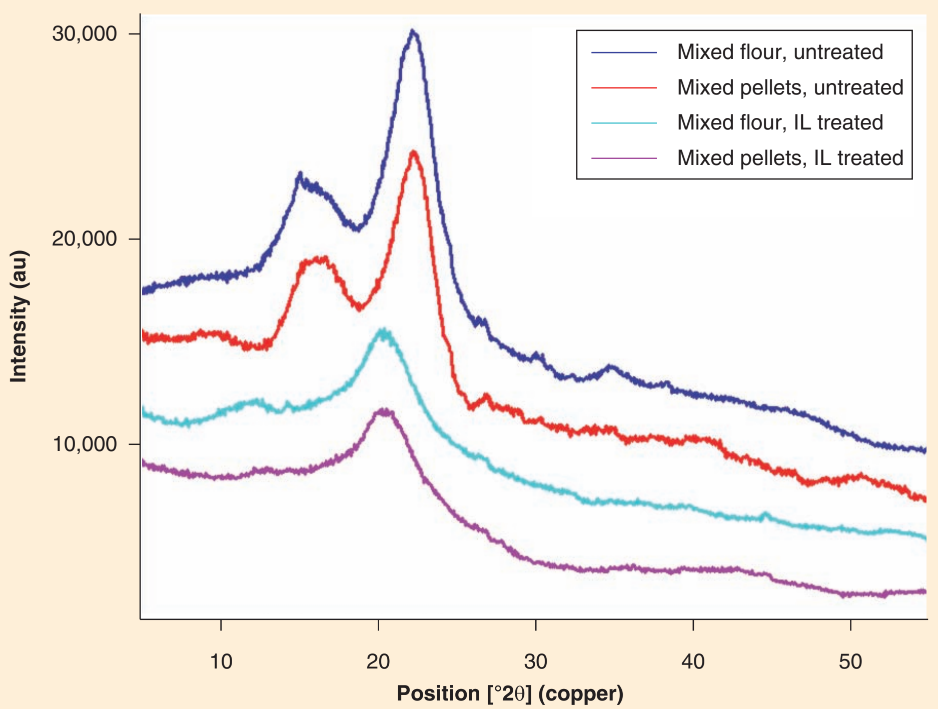

Figure 3. X-ray diffraction patterns of mixed feedstock flour and pellets before and after pretreatment with 1-ethyl-3-methylimidazolium acetate. Pretreatment conditions: biomass loading = 100 g/l, 160°C for 3 h.
IL: Ionic liquid.

Saccharification

Figure 4 indicates that IL pretreatment with [C_2mim][OAc] greatly improved the saccharification yields of both the mixed flour and pellets compared with the untreated feedstocks. For the untreated feedstocks, sugar yields of 13.6 and 19.3% were achieved with saccharification of mixed flour and pellets, respectively. The slightly higher sugar yields of mixed pellets compared with mixed flour is attributed to the pressure and heat applied on biomass feedstocks during the pelletizing process and acts as a thermochemical pretreatment. The IL-pretreated mixed pellets and flour exhibit similar high and significantly fast saccharification rates by reaching 90% digestibility in 24 h, which is well supported by previously reported studies demonstrating the equal effectiveness of IL pretreatment using [C_2mim][OAc] on various types of biomass feedstocks [23].

High yields of fermentable sugars can be realized by applying high enzyme loadings following **biomass pretreatment**, but enzyme doses need to be significantly reduced to make the conversion process commercially attractive, and pretreatment conditions and subsequent enzymatic hydrolysis must be optimized in tandem for maximum sugar release with the lowest possible amount of enzyme. We investigated the effects of enzyme loading on enzymatic hydrolysis for IL-pretreated mixed flour and pellets. **Figure 5** indicates that >80% digestibility can be achieved during 72 h enzymatic hydrolysis at an enzyme loading of 10 mg CTec2 protein/g glucan, half of the 20 mg CTec2 protein/g glucan giving >90% digestibility. However, at an even lower enzyme loading of 5 mg CTec2 protein/g glucan, much lower digestibilities of approximately 59.4 and 61.3% were achieved for mixed flour and pellets, respectively, at these high biomass loading levels. These results indicate that further studies are needed to define optimal enzyme formulations and loadings to maximize sugar yields at lower enzyme loadings for these processing conditions.

Mass balance

Figure 6 summarizes the material balances for the IL pretreatment and enzymatic hydrolysis for both mixed flour and pellets. In general, a similar mass flow and allocation was observed for mixed pellets and flour. For instance, on a 100-g basis of the raw mixed pellets, 63.1 g of pretreated solids can be recovered that retain the majority of the glucan, a portion of xylan and most of the lignin. On the same basis, 1.9 and 10.0 g of glucose and glucose oligomers, xylose and xylo-oligomers, respectively, can be recovered following posthydrolysis. Furthermore, approximately 33.3 and 6.0 g of glucose and xylose, respectively, can be recovered from the enzymatic hydrolysis of the recovered solids. The material balance generated indicates some mass loss during pretreatment and enzymatic

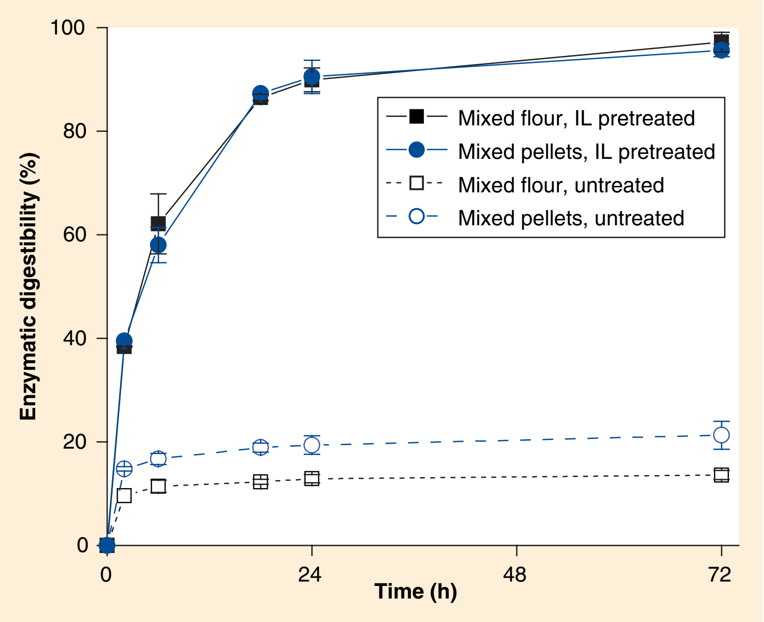

Figure 4. Comparison of enzymatic hydrolysis profiles of untreated and ionic liquid-pretreated mixed feedstock flour and pellets. Biomass loading = 20 g/l; enzyme loading 20 mg CTec2 protein/g glucan and 0.26 mg HTec2 protein/g glucan.
IL: Ionic liquid.

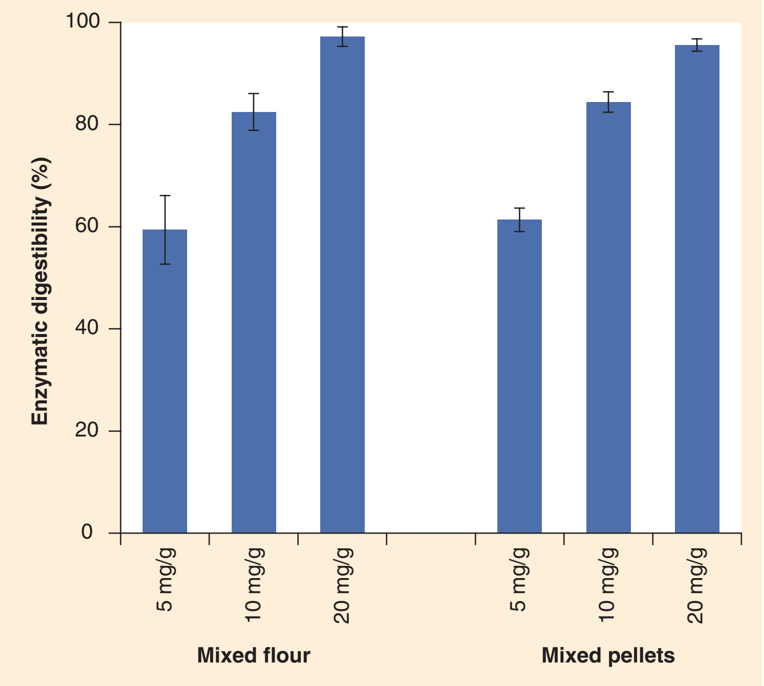

Figure 5. Comparison of enzymatic digestibility of untreated and ionic liquid-pretreated mixed feedstock flour and pellets at different enzyme loadings after 72 h of saccharification. Biomass loading = 20 g/l; enzyme loading 5, 10, 20 mg CTec2 protein/g glucan and 0.07, 0.13, 0.26 mg HTec2 protein/g glucan.

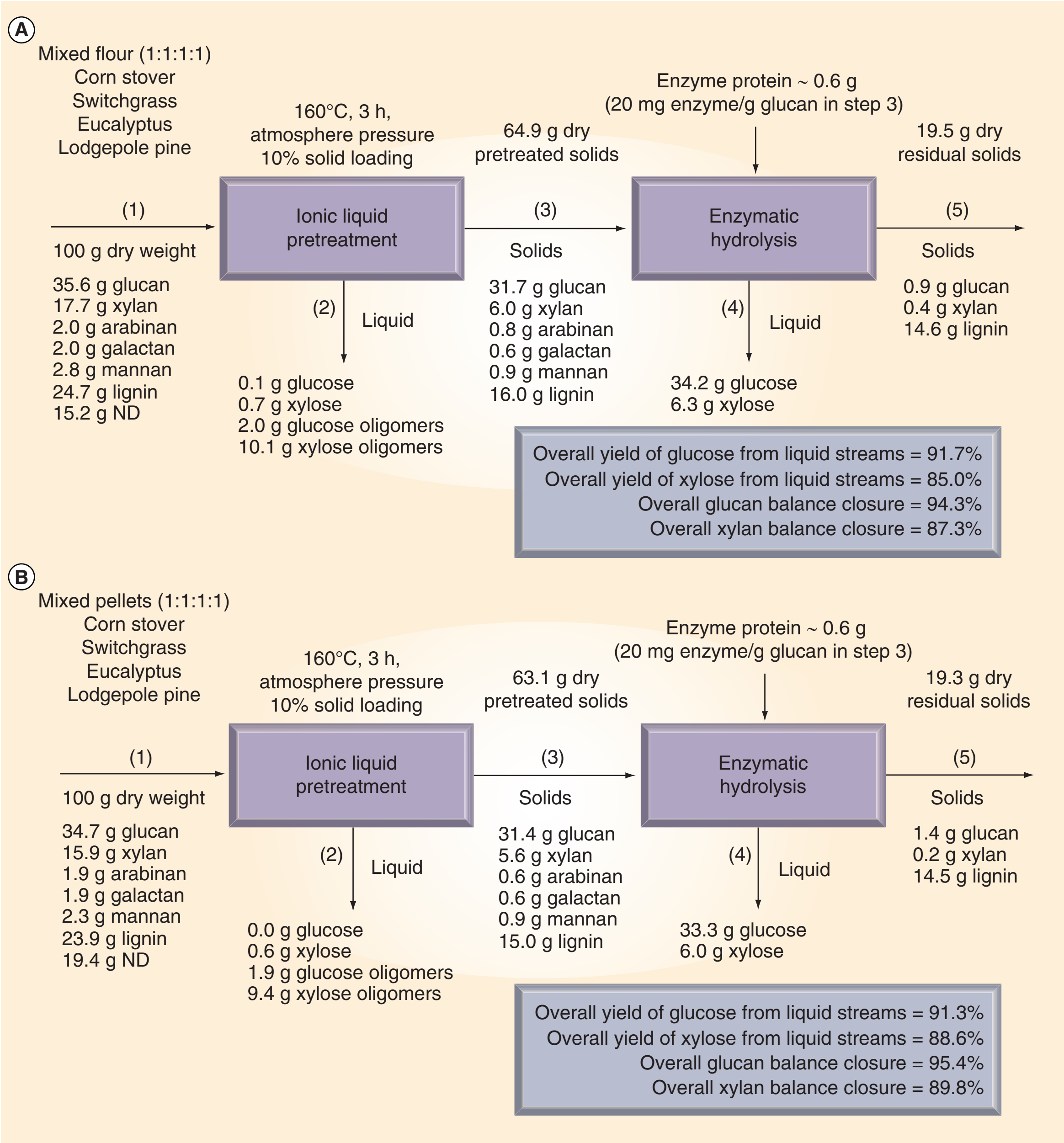

Figure 6. Partial mass balance based on parameters determined in Table 1 obtained during ionic liquid pretreatment and subsequent enzymatic hydrolysis. (A) Of mixed flour and **(B)** of mixed pellets (corn stover:switchgrass:eucalyptus:pine = 1:1:1:1 on dry weight basis). **(1–5)** indicate steps in the process. ND: Not determined.

hydrolysis, yet the overall sugar recovery in the liquid streams remains over 90%, confirming that IL pretreatment with [C_2mim][OAc] can preserve most of the sugars and substantially enhanced the effectiveness of enzymatic hydrolysis. The overall glucan closure (95.4%) was higher than xylan (89.8%), and is attibuted to the greater chemical robustness of glucose during IL pretreatment and that a fraction of the hemicellulose remained in solution after pretreatment and recovery. During pretreatment, a significant amount of lignin was also solubilized into the liquid stream; however, the residual solids after enzymatic hydrolysis are rich in lignin (>75%), indicating potential opportunities for lignin valorization. This study did not determine compounds such as proteins, sugar degradation products and lignin derivatives that may contribute to the incomplete mass balance, and additional study is needed to fully account for all components.

Conclusion

Mixed feedstocks are a potentially significant resource for the production of biofuels and biorefineries must be able to efficiently convert them into fermentable sugars with no loss of performance and/or yield. The densification of these mixed feedstocks is an effective means of increasing the energy density and enhancing the overall cost and energy balance of the entire biofuels supply and conversion chain. We have demonstrated that the IL [C_2mim][OAc] can efficiently pretreat both the mixed flour and pellets with no obvious negative impact on sugar yield and/or hydrolysis kinetics obtained from commercial enzyme cocktails. These findings indicate that mixed pellet feedstocks may be a viable and valuable resource to consider when assessing biomass availability.

Future perspective

The rate of development and deployment of advanced biofuels derived from lignocellulosic feedstocks will be dependent on several factors, with feedstock availability and affordability being among the most important. The reliance on single feedstocks to meet the tonnage requirements of any given biorefinery should be considered as high risk in terms of both availability and affordability, and mixtures of feedstocks offer a potential solution provided that they are efficiently converted into sugars and biofuels. Proper formulation and blending can provide a consistent output that minimizes the negative consequence of processing diverse feedstocks. Energy density is another critical metric for lignocellulosic biomass, and there exist several densification technologies that should be evaluated to identify the most promising approaches. The IL [C_2mim][OAc] appears to be capable of efficiently processing a wide range of feedstocks with no loss of performance, including pellets, although significant improvements in terms of cost of the IL and the need for recycling and reuse of the IL need to be resolved before this technology is commercially viable. The development of liquid–liquid extraction and advanced IL recovery techniques, such as the use of aqueous biphasic systems, offer exciting alternatives to energy-intensive distillation.

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Financial & competing interests disclosure

This work was part of the US Department of Energy (DOE) Joint BioEnergy Institute (CA, USA) (www.jbei.org) supported by the US DOE, Office of Science, Office of Biological and Environmental Research (Washington, DC, USA), through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory (CA, USA) and the US DOE (Washington, DC, USA). The US Government retains and the publisher, by accepting the article for publication, acknowledges that the US Government retains a non-exclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this manuscript, or allow others to do so, for US Government purposes. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Current limitations of feedstocks

- Biorefineries have tonnage and throughput requirements that must be met year round.
- Typically, there is no single feedstock available in any given region that is capable of meeting the price and availability demands of the biorefineries scheduled for deployment.
- Mixed feedstocks are a potential solution, but compositional variation is a challenge in terms of maintaining high conversion efficiency.
- Formulation and blending of mixed feedstocks can mitigate the impact of compositional variation.
- Energy density of biomass is relatively low, and densification of any feedstock may be required.

Ionic liquid pretreatment offers a promising route for the conversion of mixed & densified feedstocks with high sugar yields

- Ionic liquid (IL) pretreatment with certain ILs, such as 1-ethyl-3-methylimidazolium acetate, is a nascent conversion technology that has been used to efficiently convert a wide range of feedstocks.
- We have demonstrated that the IL 1-ethyl-3-methylimidazolium acetate can efficiently process mixtures of pine, eucalyptus, switchgrass and corn stover in both flour and pellet form.
- These results indicate that blending and densifying a wide range of feedstocks may be a competitive solution with no significant adverse impacts, provided that they are coupled with the appropriate conversion technology.

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