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Transforming biomass conversion with ionic liquids: process intensification and the development of a high-gravity, one-pot process for the production of cellulosic ethanol

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Abstract

Producing concentrated sugars and minimizing water usage are key elements in the economics and environmental sustainability of advanced biofuels. Conventional pretreatment processes that require a water-wash step can result in losses of fermentable sugars and generate large volumes of wastewater or solid waste. To address these problems, we have developed high gravity biomass processing with a one-pot conversion technology that includes ionic liquid pretreatment, enzymatic saccharification, and yeast fermentation for the production of concentrated fermentable sugars and high-titer cellulosic ethanol. The use of dilute bio-derived ionic liquids (a.k.a. bionic liquids) enables one-pot, high-gravity bioethanol production due to their low toxicity to the hydrolytic enzyme mixtures and microbes used. We increased biomass digestibility at >30 wt% by understanding the relationship between ionic liquid and biomass loading, yielding 41.1 g L⁻¹ of ethanol (equivalent to an overall yield of 74.8% on a glucose basis) using an integrated one-pot fed-batch system. Our technoeconomic analysis indicates that the optimized one-pot configuration provides significant economic and environmental benefits for cellulosic biorefineries by reducing the amount of ionic liquid required by ~90% and pretreatment-related water inputs and wastewater generation by ~85%. In turn, these improvements can reduce net electricity use, greenhouse gas-intensive chemical inputs for wastewater treatment, and waste generation. The result is an overall 40% reduction in the cost of cellulosic ethanol produced and a reduction in local burdens on water resources and waste management infrastructure.
Introduction

Second-generation biofuel production from lignocellulosic biomass is currently challenging as most of the processes in use are constrained by factors such as low titer and high water usage. Industrial ethanol production requires an ethanol titer of more than 40 g L\(^{-1}\) for efficient distillation.\(^1\)\(^2\) It is therefore necessary to use a high glucan loading (e.g., over 8 wt%) or use an engineered microbe that is able to efficiently convert both pentose and hexose\(^3\). High-gravity (HG) biomass processing has been frequently reported to reach this titer. For instance, with acid pretreatment followed by a water-washing step, an ethanol titer of 57 g L\(^{-1}\) was obtained with simultaneous saccharification and fermentation (SSF).\(^4\) However, that process required a large quantity of water for the removal of toxic chemicals from the pretreated biomass before saccharification.

A one-pot process has been employed in many biochemical processes because of its relative simplicity, resulting in lower operating and capital costs.\(^5\) In terms of one-pot biofuel production from lignocellulosic biomass, progress has so far been limited to the conversion of cellulose substrates, not lignocellulosic biomass. Cellulase-displaying yeast has been employed to directly ferment ethanol from cellulose.\(^6\) It was also reported that ethanol could be fermented from Solka-Floc (powdered cellulose) by using a co-culture in a one-pot process scheme.\(^7\) A recent report used a mixture of acid-pretreated (i.e., HCl) and base-pretreated (NaOH) rice straw for ethanol production, but the pretreatment had to be conducted in different reactors.\(^8\) Until now, the production of biofuels from lignocellulose using a one-pot conversion technology that includes pretreatment, saccharification, and fermentation has not been reported because of the significant technical challenges present. For example, the degradation products generated during dilute acid pretreatment (e.g., Hydroxymethylfurfural (HMF) and furfural) must be removed before enzymatic hydrolysis of pretreated biomass as HMF inhibits the enzymes used.\(^9\) In addition, the
solvents or chemicals used for pretreatment are usually toxic to the microbes and enzymes used downstream to complete the biomass conversion process, and the removal/recycle of these reaction agents can be costly.\textsuperscript{10} Because sulfuric acid used in acid pretreatment is not economical to recycle, it must be removed and disposed of using strategies that generate large quantities of solid waste or wastewater and, in some cases, result in unacceptable sugar losses or require energy- and greenhouse gas (GHG)-intensive inputs such as ammonia.\textsuperscript{11} The development of robust one-pot biomass conversion technologies operating at high solids loading can reduce biorefinery capital costs, operating costs, waste generation, and impacts on the climate and local natural resources. However, there remain engineering challenges that must be addressed before HG biomass processing could be applied using the one-pot process approach. These challenges include: 1) The mass transfer limitation that exists throughout pretreatment, saccharification, and fermentation unit operations due to the water constraint; 2) The generation of inhibitory products at high solid loading is expected and could pose problems for downstream processing,\textsuperscript{12} and concentrated end-products (e.g., glucose, cellobiose) may decrease overall enzyme activity;\textsuperscript{13} 3) Decreased viability of microorganisms due to the increased osmolarity as a result of high concentration of carbon substrates (e.g., glucose and xylose) and related end products.\textsuperscript{2}

Recently, significant progress has been made with ionic liquid (IL) pretreatment, and a one-pot process has been successfully demonstrated for biomass-sugar production that combines pretreatment and saccharification.\textsuperscript{14} The development of biocompatible and bio-derived ILs (e.g., choline-based ILs) that are proven to be effective for biomass pretreatment makes one-pot biofuel production from lignocellulose possible.\textsuperscript{15,16} We report here a one-pot HG production of ethanol using bio-derived ILs (bionic liquids). For the first time, an ethanol titer of over 40 g L$^{-1}$ from lignocellulosic biomass at $>$30 wt\% loading was achieved using an integrated fed-batch strategy with a one-pot process that combined pretreatment, saccharification, and fermentation
The resulting reduction in water consumption and improved overall process economics serve as important steps toward more affordable and sustainable second-generation biofuels.\textsuperscript{17,18}

**Results and discussion**

*Glucose profiles from bionic liquids treated corn stover*

Three choline-based ILs, including cholinium acetate ([Ch][OAc]), cholinium lysinate ([Ch][Lys]), and cholinium aspartate ([Ch]$_2$[Asp]), were compared in terms of sugar titers as well as conversion yields. Recent reports on [Ch][OAc] and [Ch]$_2$[Asp] showed high levels of lignin extraction,\textsuperscript{19,20} and another study of switchgrass pretreatment with [Ch][Lys] and [Ch][OAc] showed that over 80\% of glucose could be obtained after enzymatic hydrolysis.\textsuperscript{16}

Since pretreatment with neat IL can suffer from poor mass/heat transfer at high solids loading, IL-water mixtures were used instead for biomass pretreatment. Figure S1 presents a summary of the sugar yields after a one-pot, two-step (pretreatment and saccharification) process at different biomass loading levels. Compared to previous studies in which the ratios of biomass loading to ionic liquid loading ($R_{\text{m/i}}$) ranged from 0.05 to 0.1,\textsuperscript{16,20} the results suggest that the dilute IL pretreatment was also effective at a relatively higher $R_{\text{m/i}}$. For example, at 10\% IL levels and a $R_{\text{m/i}}$ of 0.2, [Ch][OAc] yielded 81.4\% glucose, whereas [Ch][Lys] and [Ch]$_2$[Asp] yielded over 90\% glucose. The sugar yield from [Ch][OAc] pretreatment decreased to below 70\% when the $R_{\text{m/i}}$ increased to 0.5 (Figure S1A). A successful one-pot PSF requires that the IL content in pretreatment be as low as possible, therefore it is not possible to employ a low $R_{\text{m/i}}$ (e.g., less 1) in an HS process with solid loading over 20 wt\%. The results obtained here indicated that [Ch][OAc] is not suitable for the proposed one-pot HG process. With the pretreatment using [Ch][Lys] and [Ch]$_2$[Asp], glucose yield decreased as a function of increased solids loading (Figure S1B). We attribute these results to poor mass transfer that significantly lowered
pretreatment efficiency. As shown in Figure S1B, over 80% of glucose was recovered from the initial biomass after pretreatment with [Ch][Lys] at solid loading of 34.2 wt% (equivalent to a glucan loading of 11.6 wt%). Using [Ch]$_2$[Asp], 73.9% of glucose was obtained with pretreatment at a solid loading of 29.9 wt% (equivalent to a glucan loading of 10.2 wt%).

Optimization of HS bionic liquid pretreatment: Effect of IL concentration and biomass loading on glucan saccharification

Compared to traditional neat IL pretreatment, in which IL is used for biomass dissolution (e.g., 1-ethyl-3-methylimidazolium acetate),$^{21}$ pretreatment of biomass using an IL:water mixture does not go through the process of cellulose dissolution and regeneration. We hypothesize that the lignin extraction that occurs during pretreatment using these IL:water mixtures that makes the crystalline cellulose more accessible to hydrolytic enzymes. The effect of IL concentration on HS pretreatment and saccharification was investigated. Figure S2 presents the glucose yields from both [Ch][Lys] and [Ch]$_2$[Asp] pretreatment followed by the corresponding enzymatic hydrolysis. The increase of IL loading resulted in an increase in the capacity of lignin extraction, leading to improved pretreatment efficiency as well as cellulose digestibility. The results indicate that an increase in [Ch][Lys] loading did contribute significantly to an increase in glucose yields, especially when the IL loading increased from 5 to 10 wt% (Figure S2). As the IL loading further increased to 12 wt% or 15 wt%, the hydrolysis yield did not increase proportionally. With [Ch]$_2$[Asp] pretreatment, the cellulose conversion efficiency increased with increases in IL loading. Further investigation of the IL concentration effect on fermentation was conducted and the results are discussed in the fermentation optimization section.
Response surface methodology was then employed to study how the IL loading and biomass loading together affect glucose yield after the two-step one-pot processing. Figure 1 presents modeled 3-D plots of glucose yields from corn stover pretreated with [Ch][Lys] (Figure 1A) and [Ch][Asp] (Figure 1B), and the model analysis suggests that the interaction between IL loading and mass loading was significant. As shown in Figure 1A, a [Ch][Lys] loading over 10 wt% could yield a relatively high glucose yield (> 80%) at a solid loading over 30 wt%. Further increases in IL loading did not significantly increase glucose yield at the high solid-loading level (e.g., more than 30 wt%), indicative of poor mass/heat transfer during the HS processing. It was also noticed that the corn stover was only wetted without mobile liquids (water not sequestered in the plant cell wall) when the solid loading was increased to over 40 wt% due to the hygroscopic characteristics of corn stover that limit the availability of mobile water by sequestration of water in the cell wall. For [Ch][Asp] pretreatment, further increases in IL loading (>15 wt%) increased the glucose yield to around 80% at 30 wt% of solid loading (Figure 1). This condition was then used for downstream processing.

One-pot process development for concentrated hydrolysates with fed-batch saccharification

In order to realize a robust one-pot conversion platform, a fed-batch approach is needed to achieve the desired fermentable sugar concentrations in the hydrolysates. Previous studies using high-solid water-washed steam-exploded corn stover reported 72.5% glucose yield with a sugar titer over 100 g L\(^{-1}\).\(^{12}\) In a one-pot system, however, the sugar titer and yield were limited by the solid loading used for pretreatment. In order to reach the desired sugar titer (e.g., > 80 g L\(^{-1}\) glucose) using one-pot processing, a fed-batch strategy was employed and optimized after pretreatment at 34.2 wt% solids loading at 140 °C for 3 hrs.
As shown in Figure 2, it took 6 days with 5 feeds (one initial feed plus one feed per day for the first 4 days) to reach a glucose titer of 80 g L\(^{-1}\) with strategy A. In this process, the use of water at the beginning of saccharification is important for reducing viscosity as a requirement of efficient enzymatic hydrolysis of glucan and xylan. In a continuous processing mode, the hydrolysate could be primarily used for downstream processing such as fermentation and a small portion of the hydrolysate could be used for continuous saccharification by loading more pretreated biomass. In batch mode, as is the case in this study, the use of water diluted the one-pot system and takes significantly longer time intervals to reach a concentrated hydrolysate, which is not favorable.

An improved strategy (strategy B) was to use the glucose hydrolysate from one batch of saccharification (“seed batch”, as shown in Figure 2B), in which the glucose titer was over 80 g L\(^{-1}\), as a replacement for the water used in saccharification for all the other batches (“operation batches”, as in Figure 2B). As shown in Figure 2A, with the initial loading of glucose hydrolysate, the glucose titer in each batch (e.g., Batch A in Figure 2B) was maintained at a relatively high level and it took less time (e.g., 3 days in the fed-batch mode) to reach a desired sugar titer for fermentation comparing to the time used in strategy A (Figure 2A). The improved feeding strategy was also applied for [Ch]2[Asp] pretreated corn stover, where the hydrolysate in the seed batch contained 70 g L\(^{-1}\) of glucose. As shown in Figure 2A, the sugar titer was kept around 70 g L\(^{-1}\) with one feeding per day for 6 days including additional 72 hours’ saccharification for a complete digestion of glucan. Further optimization of the fed-batch saccharification was also conducted to improve the glucose productivity by adjusting the feeding strategy. For example, the feed rate of pretreated biomass (in grams per day) was adjusted according to the digestion rate of cellulose during enzymatic hydrolysis. The results suggest that the sugar titer could be
maintained after increasing the feed rate by 50%, which results in a 50% increase in terms of glucose productivity.

It was previously reported that an air-drying process could lower the moisture content in the pretreated slurry, with a corresponding increased in glucose titer, but it is unknown whether or not the drying process might change biomass structure (e.g., porosity) and further affect cellulose digestibility and/or if the resulting concentrated IL would affect fermentation efficiency. The energy consumption associated with air-drying is also an issue that prevented its use in this study. It is also worth mentioning that end-product inhibition (e.g., concentrated glucose and cellobiose) could affect the enzyme activity and further lower glucose yield. Simultaneous saccharification and fermentation was thus incorporated into the one-pot system to improve the overall yield of glucose as well as ethanol.

Towards sustainable bioethanol production using one-pot HG process

Simultaneous saccharification and fermentation is a frequent practice for cellulosic ethanol production, which is favored to reduce end-product inhibition of enzymatic hydrolysis and increase productivity. Previous studies using SSF reported successful ethanol production from cellulosic biomass. Since the optimized temperature for enzymatic hydrolysis (e.g., 50 °C) and yeast-ethanol fermentation (e.g., 30 °C when using wild type yeast) are different, developing a controlled temperature strategy is critical for a successful high-solid fed-batch SSF. For example, a recent study using delayed SSF, in which the initial temperature was 45 °C for 12 hours pre-saccharification and was then cooled to 30 °C for SSF, showed improved yield and productivity. Constant temperature (~37 °C) has also been used for high solid fed-batch SSF from sugarcane bagasse. In
order to increase fermentation productivity, it is imperative that the substrate viscosity be reduced at the early stage of SSF. Pre-saccharification at 50 °C for 24 hours was employed after feeding all the HS content biomass slurry. The effect of temperature on the performance of fed-batch SSF (FB-SSF) was then investigated at a yeast inoculation of 0.2%. Two different temperatures, 30 °C and 37 °C, were compared after the pre-saccharification stage. The results show that the FB-SSF at 37 °C yields 71.6 % of ethanol, which is higher than at 30 °C (67.1%) in 72 h. A compositional analysis of the residue after fermentation showed that 13.7 % of cellulose was remained at 30 °C, whereas only 10.2 % of cellulose was remained at 37 °C. This difference in undigested cellulose indicates that the low conversion yield is due to the fact that the saccharification rate was lower at a relatively low temperature (30 °C).

Yeast loading was also investigated, as shown in Figure 3A. Previous study of SSF using relatively low solid-loading biomass (~ 10%) suggested an optimal yeast loading of 1-2 g L\(^{-1}\) yeast cell \(^{26}\). In the current study, the ethanol yield was lower when using 1 g L\(^{-1}\) than that using higher yeast loading, and that ethanol fermentation was incomplete (at 72 hr) when the yeast loading was below 1 g L\(^{-1}\) (data not shown). This indicates that the low yeast loading resulted in stuck fermentation. Figure 3A also suggests that there is no significant difference in ethanol yield when the yeast loading increased from 3 to 5 g L\(^{-1}\). In addition, when the biomass feeding amount was doubled in FB-SSF, the ethanol yield and titer were 41.1 g L\(^{-1}\) and 74.8 %, respectively (Figure 3B), indicating that the one-pot process is stable at higher biomass loading levels and that the process of continuous feeding is possible. In the case of the batch process, the ethanol productivity was 0.7 g L\(^{-1}\) h\(^{-1}\) during the first 48 h and then decreased because of the depletion of glucose after 48 h.
As discussed previously, increasing the [Ch]<sub>2</sub>[Asp] concentration to over 10 wt% during pretreatment led to an increased glucose yield. As shown in Figure 4, the [Ch]<sub>2</sub>[Asp] concentration played an important role for the one-pot ethanol fermentation. The increase of [Ch]<sub>2</sub>[Asp] concentration in pretreatment significantly decreased the ethanol yield to about 50%, and the residual glucose suggested that the fermentation was incomplete at 96 h because of the low productivity. The decrease in ethanol yield could be due to the increased osmolarity that might lead to cell shrinkage and decreased cell viability. Increases in yeast loading increased ethanol yield at the elevated [Ch]<sub>2</sub>[Asp] loading (15 wt%) (Figure 4). At the same solids loading (29.9 wt%), increasing the yeast loading to 0.7% yielded 72.2% of ethanol (34.2 g L<sup>-1</sup>). However, further increases in solids loading generated lower ethanol yields.

Figure 5 shows a comparison of different scenarios. By eliminating the washing and solid/liquid separation steps, the one-pot process results in minimized water usage as low as 3 kg/kg of biomass. Our glucan/glucose balance suggests that over 90% of glucose from saccharification has been converted to ethanol, yielding an overall conversion of 74.8% in one-pot. As a result, 144.8 g ethanol was produced from the glucan present in 1 kg of corn stover. The one-pot system of fed-batch SSF could be enhanced for continuous ethanol fermentation with minimal modification. Besides the yeast-ethanol fermentation, the concentrated sugar stream from the HS fed batch process also provides flexibility for the other types of microbial conversion, which make it possible to convert for a broad range of fuels or chemicals at a relatively high titer in one pot. Integrated biomass processing strategies could be developed depending on the compatibility of IL and microbes as well as the downstream recovery pathway. For example, in situ product recovery (e.g., gas stripping)<sup>27</sup> could be applied to the fed batch system for continuous
production of butanol. In addition, the utilization of xylose in the hydrolysates could generate a more cost efficient process. For example, a microorganism that is capable of converting both glucose and xylose could utilize this concentrated sugar stream for improved biofuel yield.²⁸

*Production cost analysis*

One-pot HG processing can significantly reduce the ethanol production cost compared to the conventional IL pretreatment (e.g., 1-ethyl-3-methylimidazolium acetate) of biomass, as shown in Figure 5. Previous techno-economic analyses of cellulosic ethanol production with IL pretreatment²⁹,³⁰ have identified the IL/biomass ratio as a critical factor that affects the minimum ethanol selling price (MESP) and concluded that the ratio must be below 2 to achieve an MESP below $5 gal⁻¹. The use of dilute IL (e.g., 10 wt% of [Ch][Lys]) for biomass pretreatment in the current one-pot configuration reduced the usage of IL by decreasing the ratio from approximately 3.6 to 0.3. Consequently, the cost incurred due to unrecovered IL was much lower in the current one-pot process. The use of cholinium-based IL may also reduce cost because it can be synthesized from renewable sources, namely choline-hydroxide and lysine, using very straightforward processing and minimal separations. Another important factor that typically limits the large-scale IL processing of cellulosic biomass is the quantity of water required during production. Similar to the other pretreatment technologies, conventional IL pretreatment requires a detoxification step to remove IL and other inhibitors that are harmful for downstream saccharification and fermentation. The conventional IL process also requires an anti-solvent (e.g., water) for cellulose regeneration. This introduces additional processing
steps such as water washing, filtration, and wastewater treatment. The use of a one-pot PSF strategy eliminates these steps and thus reduces capital and operating costs.

As shown in Figure 5, the water usage in the current HG configuration is reduced by greater than 85% relative to the conventional IL process, which reduces operating expenditures in the pretreatment, wastewater treatment, and cogeneration sections (Figure S5). The cost analysis as described in the methods section showed that the current one-pot HG process has the potential to reduce the annual operating cost (AOC) by more than 40% (Figure 5). A cost analysis of co-fermentation using both glucose and xylose for ethanol production was also modeled and compared (See Supporting Information). The results of this projected co-fermentation case suggest that the MESP could be further reduced to approximately $2.8 gal⁻¹ (2014 USD).

Conclusions

For the first time, cellulosic ethanol was produced at a titer of over 40 g L⁻¹ in an optimized one-pot PSF process. The use of dilute bionic liquids enabled efficient pretreatment of lignocellulosic biomass at a solid loading as high as 34.2 wt%, yielding over 80% glucose in one pot. The integrated one-pot PSF process combined with an improved feeding strategy effectively improved mass transfer without a dilution of the system and is able to continuously provide a concentrated glucose stream for ethanol production at high titer. The optimized ethanol yield and titer were 74.8% and 41.1 g L⁻¹, respectively. Benefiting from the high solid feeding strategy, the one-pot process significantly reduced water usage from up to 20 kg/kg corn stover in a conventional water-wash process to just 3 kg/kg (an 85% reduction) in a single vessel without intervention or clean-up. In a biorefinery utilizing water recycling, the one-pot process provides substantial economic benefits through reduced IL inputs and wastewater
generation. The resulting reductions in water demand, wastewater brine disposal, and energy-intensive chemical inputs have the potential to reduce GHG emissions and alleviate local environmental burdens. Compared to the conventional IL process, the economic analysis suggested that the current configuration could reduce the AOC by 40% (Figure 5) with significant cost savings in terms of the MESP. These results establish a new approach to affordable, sustainable, and scalable biomass conversion using ionic liquids based on process intensification and integration.

**Experimental**
All of the chemicals were reagent grade and purchased from Sigma-Aldrich (St. Louis, MO) if not specified. The enzymes (Cellic® Ctec 2 and Htec 2) were given by Novozymes North America (Franklinton, NC), containing 188 mg protein per mL. Corn stover was supplied by the Department of Chemical Engineering & Materials Science at Michigan State University. The biomass was ground by a Thomas-Wiley Mini Mill fitted with a 20-mesh screen (Model 3383-L10 Arthur H. Thomas Co., Philadelphia, PA, USA) and analyzed for polysaccharide composition (glucan 34.1 wt% and xylan 25.1 wt%). Cholinium Acetate ([Ch][OAc]) was purchased from Sigma and used as received. Cholinium Lysinate ([Ch][Lys]) and Choline Aspartate ([Ch]₂[Asp]) were synthesized as reported.¹⁶,²⁰

*Novel dilute bio-derived ionic liquid pretreatment*

The pretreatment was conducted in 50-mL pressure tube (Ace Glass Inc., Vineland, NJ, USA). In a typical HS pretreatment (e.g., 30 wt%), for example, 3 g of corn stover was loaded in 10 g of IL/water solution with a certain IL concentration (e.g., 10 wt%). After a thorough mixing of IL, water, and biomass, the tube was submerged in an oil bath at 140
°C for 3 hours. The solid loading amount in this study is presented as a percentage ratio of dry biomass weight (g) to the weight of IL/water mixture (g). After pretreatment, the slurry was cooled down to room temperature and the pH was adjusted to 5 by thoroughly mixing with hydrochloric acid before saccharification.

**Enzymatic saccharification**

The saccharification was carried out at 50 °C and pH 5 at 48 rpm in a rotary incubator (Enviro-Genie, Scientific Industries, Inc.) using commercial enzyme mixtures, Cellic® CTec2 and HTec2, with an enzyme dosage of 20 mg protein per gram glucan and 2 mg protein per gram xylan, respectively. One-pot processing was employed and no IL separation was conducted. For the optimization of glucose yield, the one-pot process was conducted with additional water during saccharification for improving mixing and the solid content was around 10 wt%. In order to provide concentrated hydrolysates, fed-batch process was conducted depending on the solid loading used in pretreatment. For example, with a basic feeding strategy (strategy A), 11.2 g pretreated biomass slurry at solid loading of 34.2 wt% was separated into 3 loads (e.g., 3.5 g, 3.5 g, and 4.2 g) for loading every 24 hrs in 2 days into 4 mL initial solution (e.g., water). With an improved feeding strategy (strategy B), the initial water solution was replace with concentrated glucose solution (e.g., 80 g L⁻¹) from an independent batch (“seed batch”, as shown in Figure 2), and pretreated biomass was continuously loaded into the seed batch for supplying hydrolysates to operation batches (e.g., batch A, B & C). Citric acid buffer (pH 5, 40 mM) was added to maintain the pH during the optimization.

**Fermentation**

*Saccharomyces cerevisiae* strain BY4741 (MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0), a derivative of S288C was activated according to NREL procedure ³¹. Yeast inoculation
was initiated with the concentrated hydrolysates directly from saccharification. For an integrated one-pot ethanol SSF, the temperature was decreased after a 24 hours’ pre-saccharification (50 °C), and the SSF was then conducted in an anaerobic condition at 120 rpm with specified temperature.

**HPLC analysis**

In order to accurately determine the ethanol and sugar yield, the current study employed a reported method, in which the slurry sample was diluted extensively (at least 10 times) and then measured by HPLC (Agilent HPLC 1200 Series) equipped with a Bio-Rad Aminex HPX-87H column and a Refractive Index detector. The solid fraction after saccharification or fermentation in a dilute solution is below 1 wt% after dilution and its volume displacement could then be negligible. The glucose yield is represented as a percentage of the initial glucose content in corn stover before processing; likewise, the ethanol yield is represented as a percentage of the theoretical amount from the initial glucose content in corn stover (e.g., theoretically, 0.511 gram ethanol per gram glucose).

**Techno-economic analysis**

To carry out the TEA, a detailed biorefinery model developed in SuperPro designer was used in this study (Table S1, ESI†). The biorefinery model encompasses pretreatment, hydrolysis, fermentation, product recovery, wastewater treatment, and an onsite cogeneration facility. The plant was designed to process 2000 dry MT/day and most of the process and economic data were taken from a recent study by National Renewable Energy Laboratory (NREL) 11. Consistent with the NREL study, the minimum ethanol selling price (MESP) was computed based on a detailed cash flow analysis with a 10% internal rate of return. The base year for economic analysis in the current study is 2014. In order to benchmark the economic performance of the one-pot HG process, a conventional IL
process that involves a water-washing (WW) step prior to enzymatic hydrolysis was used as a reference scenario \(^{30}\) (Figure S3). Unlike the choline-based ILs used in the one-pot HG process, the WW process used 1-ethyl-3-methylimidazolium acetate, which is not compatible with commercial enzymes. Hence most of the IL (>99.9%) was removed from the pretreated biomass using a water-intensive water-wash step. In an optimized WW process configuration with water recycling, water loading in the water-wash step (i.e., mass ratio between water used and biomass) could be as high as 20. The one-pot HG process using \([\text{Ch}][\text{Lys}]\) was considered for comparison. For both of these processes, high IL recovery (>99.9%) was assumed, using pervaporation technology detailed in Figures S3 and S4 (process flow diagrams for WW and one-pot configurations, respectively). To capture the economic merits of the one-pot process (Figure S4), three process scenarios were constructed: one conventional scenario with co-fermenting microbes and two one-pot HG scenarios (without and with co-fermenting microbes, labelled as ‘current’ and ‘projected’ scenarios, respectively) (Figure 5).

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References


Figure captions.

Figure 1. 3-D plots of glucose yields after one-pot pretreatment and saccharification. (A) Yields with [Ch][Lys] pretreatment; (B) Yields with [Ch]$_2$[Asp] pretreatment.

Figure 2. Fed-batch high-solid saccharification of ionic liquid pretreated corn stover. (A) Glucose profiles with two fed-batch strategies (■: Feeding [Ch][Lys] pretreated corn stover with strategy A; ▲: Feeding [Ch][Lys] pretreated corn stover with strategy B; ●: Feeding [Ch]$_2$[Asp] pretreated corn stover with strategy B. The concentration was sampled and measured right before each feeding.); (B) Illustration of fed-batch strategy A&B.

Figure 3. Process optimization of one-pot high-gravity ethanol fermentation after [Ch][Lys] pretreatment. (A) Effect of yeast loading on ethanol fermentation; (B) Illustration of the glucose consumption and ethanol production during simultaneous saccharification and fermentation in the one-pot system.

Figure 4. Ethanol yield of [Ch]$_2$[Asp] pretreated corn stover with increasing yeast inoculation (0.3%, 0.5%, 0.7%, and 0.9%). Case 1: as reference, using 10% (in weight, same as below) of ionic liquid and 29.9% of biomass loading; Case 2: using 15% of ionic liquid and 29.9% of biomass loading; Case 3: using 15% of ionic liquid and 34.2% of biomass loading.

Figure 5. Comparison of three scenarios in terms of water loading, ionic liquid (IL) loading, annual operating costs (AOC), and minimum ethanol selling price (MESP). Scenario 1. Conventional ionic liquid process, including a water-washing step before simultaneous saccharification and fermentation (SSF); Scenario 2. Current one-pot high-gravity (HG) PSF (pretreatment, saccharification, and fermentation) configuration for ethanol production from glucose; Scenario 3. Projected system based on the current one-pot high-gravity configuration plus co-fermentation of ethanol from both glucose and xylose.
Figure 1. 3-D plots of glucose yields after one-pot pretreatment and saccharification. (A) Yields with [Ch][Lys] pretreatment; (B) Yields with [Ch][Asp] pretreatment.
Figure 2. Fed-batch high-solid saccharification of ionic liquid pretreated corn stover. (A) Glucose profiles with two fed-batch strategies (■: Feeding [Ch][Lys] pretreated corn stover with strategy A; ▲: Feeding [Ch][Lys] pretreated corn stover with strategy B; ●: Feeding [Ch]₂[Asp] pretreated corn stover with strategy B. The concentration was sampled and measured right before each feeding.); (B) Illustration of fed-batch strategy A&B.
Figure 3. Process optimization of one-pot high-gravity ethanol fermentation after [Ch][Lys] pretreatment. (A) Effect of yeast loading on ethanol fermentation; (B) Illustration of the glucose consumption and ethanol production during simultaneous saccharification and fermentation in the one-pot system.
Figure 4. Ethanol yield of $\text{[Ch]}_2[\text{Asp}]$ pretreated corn stover with increasing yeast inoculation (0.3%, 0.5%, 0.7%, and 0.9%). Case 1: as reference, using 10% (in weight, same as below) of ionic liquid and 29.9% of biomass loading; Case 2: using 15% of ionic liquid and 29.9% of biomass loading; Case 3: using 15% of ionic liquid and 34.2% of biomass loading.
Figure 5. Comparison of three scenarios in terms of water loading, ionic liquid (IL) loading, annual operating costs (AOC), and minimum ethanol selling price (MESP). Scenario 1. Conventional ionic liquid process, including a water-washing step before simultaneous saccharification and fermentation (SSF); Scenario 2. Current one-pot high-gravity (HG) PSF (pretreatment, saccharification, and fermentation); Scenario 3. One-pot HG process using co-fermentation.
configuration for ethanol production from glucose; Scenario 3. Projected system based on the current one-pot high-gravity configuration plus co-fermentation of ethanol from both glucose and xylose.