Predictive modeling to de-risk bio-based manufacturing by adapting to variability in lignocellulosic biomass supply

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HIGHLIGHTS

- Feedstock blending can enable nationwide production of biofuels.
- Predictive model can identify ideal blend ratios to achieve high sugar yields.
- A low ratio of high-quality feedstock can substantially improve sugar yields.

GRAPHICAL ABSTRACT
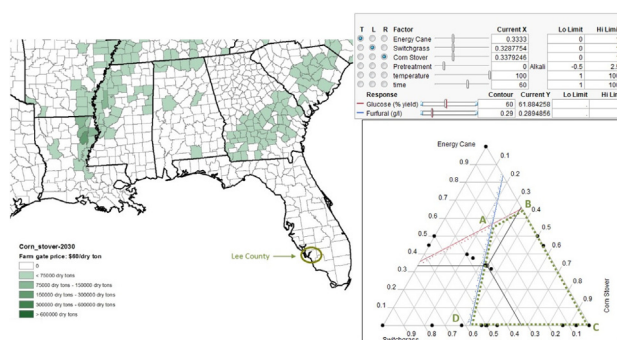
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ABSTRACT

Commercial-scale bio-refineries are designed to process 2000 tons/day of single lignocellulosic biomass. Several geographical areas in the United States generate diverse feedstocks that, when combined, can be substantial for bio-based manufacturing. Blending multiple feedstocks is a strategy being investigated to expand bio-based manufacturing outside Corn Belt. In this study, we developed a model to predict continuous envelopes of biomass blends that are optimal for a given pretreatment condition to achieve a pre-determined sugar yield or vice versa. For example, our model predicted more than 60% glucose yield can be achieved by treating an equal part blend of energy cane, corn stover, and switchgrass with alkali pretreatment at 120 °C for 14.8 h. By using ionic liquid to pretreat an equal part blend of the biomass feedstocks at 160 °C for 2.2 h, we achieved 87.6% glucose yield. Such a predictive model can potentially overcome dependence on a single feedstock.

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1. Introduction

Petroleum industry has long been utilizing the power of statistical modeling to rapidly adapt process conditions per compositional variability of incoming crude oil feedstocks and manufacture a variety of products. Feedstock composition of each batch of crude oil shipped to a refinery can vary considerably

and this variation becomes pronounced because a commercial-scale petroleum refinery processes crude oil in quantities exceeding 100,000 barrels per day (EIA, 2016). This variability can make any attempts at pre-determining optimal process conditions futile. Petroleum refineries utilize non-linear modeling to tune process conditions and fully convert each batch of crude oil but, in consequence, vary the yields of individual products existing in their pre-established suite (Hsu and Robinson, 2007; Hu et al., 2002). Complete conversion of feedstock has a higher impact on process economics than variability in quantities of products manufactured by a refinery. Furthermore, a refinery's ability to adapt to feedstock variability mitigates risks associated with dedicated feedstock supply chains.

The ability to efficiently and economically utilize variable and multiple biomass feedstock streams comprising of agricultural residue, energy crops, and municipal solid waste is critical to growing the biofuel/ biochemical sectors to the billion-ton scale, beyond the pioneer bio-refinery plants with dedicated biomass feedstocks (Langholtz et al., 2016). This is especially true given the 1) impact of annual and geographic variation on biomass yields and types (Emerson et al., 2014) and 2) process changes, relative to cellulosic ethanol, needed to deploy a slate of products, including hydrocarbons and lignin-derived compounds (Langholtz et al., 2016). Integrating biomass such as energy crops into existing crop rotations and blending these feedstocks with existing wastes and residues available to a bio-refinery will de-risk the logistics of biomass supply (Lamers et al., 2015; Langholtz et al., 2016; Li et al., 2016; Ray et al., 2017; Williams et al., 2016). In contrast to this approach, pioneer bio-refineries in Iowa, Kansas, etc. have been optimized to process a single feedstock such as the agricultural residue, corn stover (Evans, 2014). Even at these pioneer sites, to hedge supply-side risks and enable farmer choice in which crops to grow in a given season, it is likely that commercial biofuel producers will need to diversify feedstock inputs.

Idaho National Laboratory (INL) is currently developing a method to blend high-quality feedstocks with low-quality ones to reduce supply-side risks at commercial-scale biorefineries. High-quality feedstocks are defined as those that deconstruct and convert readily to biofuels. Least Cost Formulation (LCF) is being developed to evaluate low-cost biomass feedstocks across the United States and identify geographical locations for bio-refineries that can integrate diverse biomass feedstocks from multiple sources into their supply chain and lower overall feedstock costs (Sun et al., 2015). While such integration is theoretically possible, it is vital to ascertain that blending feedstocks does not negatively impact biomass conversion performance, and thereby product yields. Our understanding of biomass deconstruction, a process central to bio-refining process chain, has proliferated over the past century, but most of these studies were conducted on a single biomass feedstock (Dreyfus, 1936; Elander et al., 2009; Faith, 1945; Lloyd and Wyman, 2005; Wyman et al., 2005a,b). Very little research has been conducted on breaking down biomass blends with traditional acidic and basic catalysts (Brodeur-Campbell et al., 2012; Ewanick and Bura, 2011; Vera et al., 2015). Recent studies have shown that some novel catalysts, specifically ionic liquids (ILs), have the potential of breaking down biomass blends with minimal loss of performance in terms of sugar yields (Li et al., 2017; Shi et al., 2013). Bio-refineries, whether applying traditional or novel deconstruction catalysts, will have to be equipped with the ability to tune deconstruction processes per compositions of biomass blends and completely convert them to valuable intermediates and products. Blending feedstocks and tuning deconstruction process per the composition of biomass blends will be vital in attaining the goal of de-risking bio-refining.

Advanced Biofuels Process Demonstration Unit (ABPDU) at the Lawrence Berkeley National Laboratory (LBNL) collaborated with INL and Sandia National Laboratory (SNL) to embark upon a multi-year project to strengthen the emerging feedstock conversion interface and improve economics of deconstruction of biomass blends and their conversion to bio-products. The goals of this study were to (i) use the LCF model to select three diverse types of biomass feedstocks available in a geographic region and (ii) build a robust predictive model to maximize sugar yields by identifying optimal deconstruction process conditions for a given biomass blend or vice versa. Other forms of renewable energy producing systems, including solar and wind, have long been developing and applying predictive models to accept varied levels of feed input (Al-Amoudi and Zhang, 2000; Chow and Leung, 1996; Hatziargyriou et al., 1993; Landberg, 1999). Developing a predictive model on biomass deconstruction and integrating it to the established LCF model allows for a robust and sustainable platform for commercial-scale bio-based manufacturing.

2. Materials and methods

2.1. Biomass feedstocks and compositional analyses

LCF, a modeling system based on data from three different sources: Billion Ton Update, Biomass Logistics Model, and Bioenergy Feedstock Library, was used to select feedstocks for this study; see Section 3.1 for further details. The LCF software, developed in AnyLogic™, accesses the grower payment/access costs, logistics costs, and feedstock quality characteristics respectively, from each of the sources and identifies the locations that are conducive to establishing a commercial bio-refinery (Sun et al., 2015). The feedstocks chosen for this study included: Energy Cane (EC), Switchgrass (SG), and Corn Stover (CS) and were provided by Idaho National Laboratory located in Idaho Falls, Idaho. CS and SG were harvested from Palo Alto, IA and Texas County, OK in October 2011 and October 2010, respectively. EC was harvested from Oktibbeha County in Mississippi and provided by Mississippi State University in 2012. The feedstocks were processed at INL's Feedstock Process Demonstration Unit as part of DOE's Biomass Feedstock National User Facility in Idaho Falls, ID. The feedstocks were stored in plastic drums in a cold room at 4 °C and brought to room temperature (~25 °C) four hours prior to further size reduction with a Model No. 4 Thomas Wiley Mill. The milled feedstocks were sieved to isolate biomass particles between 20 and 80 meshes (0.85 mm and 0.18 mm, respectively) using a sieve shaker (Vibratory Sieve Shaker AS 200, Retsch, Newtown, PA, USA). The 20–80 mesh feedstocks were washed with hot water (80 °C) to remove free sugars and completely dried in an oven at 45 °C prior to compositional analysis. Whereas, the compositional analyses of individual biomass feedstocks were determined using a NREL laboratory analytical protocol (see Table 1), the composition of feedstock blends were calculated based on the weighted averages of each of the feedstocks (Hames et al., 2008; Sluiter et al., 2008a, b). The moisture content (%) of each feedstock was determined by heating them individually to 105 °C in moisture content analyzer (Mettler Toledo, Model HB43-S Halogen) until a steady state mass was achieved. The loss of weight was attributed to moisture content and was calculated to be 12.4%, 9.1%, and 14.3% for EC, SG, and CS, respectively. Moisture contents of blends were also measured to confirm that they are consistent with the calculated weighted averages of the moisture content acquired from individual feedstocks, per their mass ratios in the blends.

2.2. Pretreatments

Deconstruction of biomass was conducted in a two stage process: chemical pretreatment followed by enzymatic hydrolysis.

Table 1
Feedstock composition before pretreatment.

Feedstock[a]	Glucan (%)	Xylan (%)	Lignin (%)
Energy cane	34.64	23.65	29.13
Switchgrass	35.40	23.98	23.24
Corn Stover	33.86	15.00	24.96

[a] Values represent the average of each component based on dry feedstock.

Three different pretreatment catalysts: dilute acid, dilute alkali, and ILs were used in the form of 1% (w/w) sodium hydroxide in water (Sigma Aldrich, St. Louis, MO), 1% (w/w) sulfuric acid in water (Sigma Aldrich, St. Louis, MO), and 1-ethyl-3-methyimadozolium acetate with more than 99% purity ([C2mim][OAc]) (BASF, Ludwigshafen, Germany), respectively. Feedstocks and their blends were not washed prior to pretreatment experiments. In the case of dilute alkali and acid pretreatments, 1.0 g (dry weight) of biomass blends were fed to tube reactors (0.75″ diameter, 0.065″ thickness, 5.9″ length) followed by aqueous sulfuric acid (1% w/v) or sodium hydroxide (1% w/v) to reach a total weight of 10 g. The tube reactors were made of stainless steel (SS316L) and procured from Alloy Metal & Tubes (Houston, TX). The slurries were then thoroughly mixed in the tube reactors prior to overnight soaking. In the case of IL, about 30 min prior to pretreatment, biomass blends were mixed with the catalyst in glass tubes at the same solids loading of 10% (g dry untreated biomass/g slurry).

The tube reactors in dilute acid and alkali pretreatments were sealed with 316 Stainless steel Swagelok caps (Fremont, CA) with maximum allowable working pressure of 3300 psi. Sealed tube reactors were placed in a porous metal basket and lowered into a fluidized sand bath (Omega Engineering, Stamford, CT) maintained at the predetermined reaction temperature (Lloyd and Wyman, 2003, 2005). The temperature of the tube reactors were monitored using a data logging hand held thermometers (Mc Master-Carr, CA) welded to a single tube reactor filled with 10 g of deionized water. A stopwatch was used to measure the time taken to heat the welded tube reactor to the predetermined reaction temperature (±2.5 °C) and another stopwatch to measure the duration of reaction time, which varied per the treatments in experimental design. Upon achieving the reaction time, the tube reactor assigned to the particular treatment was pulled out of the sand bath and plunged into an ice bath for rapid cooling to <80 °C in less than 5 min. All tube reactors were then stored at 4 °C overnight, prior to enzymatic hydrolysis. To maintain consistency across all pretreatments, we stored the tube reactors after pretreatment for about 12 h in the refrigerators. Post refrigeration, the entire contents of the tube reactors were transferred to a 50 ml Erlenmeyer flask. To recover all the insolubles of the slurry, the tubes were washed with a total volume of 7.5 ml sodium citrate buffer (0.1 M). In case of some alkali pretreatments, the reaction temperatures were lower than 100 °C and reaction times longer than 12 h. In such cases, the tube reactors were placed in a convection oven (Binder, Bohemia, NY) for the duration of experiments followed by rapid cooling, storage, and recovery.

IL pretreatments were conducted in 15 ml glass pressure tubes (Ace Glass Inc, Vineland, NJ, USA) that were heated in an oil bath (Li et al., 2011, 2010). Since IL pretreatments are conducted at atmospheric pressures, ace thread and back seal caps were sufficient to seal them. Throughout the IL pretreatment, slurries were agitated using a micro-stir bar and a magnetic stirring plate. Once the reaction time was achieved, glass tubes were removed from the oil bath and pretreated slurries were then transferred to centrifuge tubes (50 ml). Washing of IL pretreated slurries was conducted in centrifuge tubes by adding 30 ml hot deionized water (~70 °C) and vigorously shaking the slurry for 15 min. The supernatant was decanted and the washing process was repeated five

times in order to remove residual IL from pretreated biomass. The pretreated solids were stored in a refrigerator, prior to enzymatic hydrolysis.

2.3. Enzymatic saccharification and HPLC analysis

Enzymatic hydrolysis was conducted at 4% (w/w) solid loading with Ctec2® and Htec2® (Novozymes, Davis, CA) enzyme loading at 10 or 40 and 1 or 4 mg protein/g glucan in untreated feedstock blends, respectively. The protein concentrations of the two enzymes, CTec2 and HTec2, as determined by the Bradford assay (Bio-Rad, Hercules, CA) with bovine serum albumin standard, were 190 and 174 mg/ml, respectively. It is important to reiterate that we did not separate pretreated solids from the liquor. We performed enzymatic hydrolysis on the entire slurry, as it is more relevant to a commercially-viable, process intensified technologies (Krishna et al., 1999, 1998; Vincent et al., 2014). Prior to enzyme application, 2% (w/w) sodium azide (0.2 ml) was added to all slurries and pH adjusted to 5.5 with either sulfuric acid (72.0%, Sigma-Aldrich, St. Louis, MO) or sodium hydroxide (50%, Sigma-Aldrich, St. Louis, MO). As explained in Section 2.2, 0.1 M sodium citrate buffer (7.5 ml) was added to the tube reactors when recovering dilute acid and alkali pretreated solids. The buffer was added separately, prior to enzyme addition, when hydrolyzing IL pretreated feedstocks. Deionized water was then added directly to all slurries to obtain a total weight of 25 ml in 50 ml Erlenmeyer shake flasks. All flasks were maintained at 50 °C in a shaking incubator (Max Q 8000, Thermo Scientific, Waltham, MA) for 120 h and samples were taken periodically at time intervals of 1, 12, 24, 72, and 120 h. These samples were centrifuged at 18,000 RCF for 30 min in a vial with a 0.2 μm filter and stored in a −20 °C freezer prior to HPLC analysis.

Dionex HPLC (Thermo Scientific, Waltham, MA) with an Aminex HPX-87H® analytical column (7.8 × 300 mm) (Bio-Rad, Hercules, CA) was used to analyze pretreatment and enzymatic hydrolysis liquid samples for glucose, xylose, furfural, acetic acid and 5-hydroxymethylfurfural (HMF). The column was maintained at 65 °C while the refractive index detector was maintained at 50 °C. A 5 mM sulfuric acid eluent was used at a flow rate of 0.6 ml/minute for this 50-min method. Dionex Chromeleon® software was used to identify peaks of the analytes and integrate the area under peaks to calculate concentrations, relative to known standards. Standards for HPLC, such as glucose, xylose, acetic acid, furfural, and HMF were purchased from Sigma-Aldrich (St. Louis, MO).

Glucose yield (%)

$$= \frac{\text{Measured glucose concentration (g/L)} \times \text{Total EH volume}(0.025\text{L}) \times 0.9}{\text{Total theoretical glucan (g) in initial biomass}} \times 100 \tag{1}$$

Xylose yield (%)

$$= \frac{\text{Measured xylose concentration (g/L)} \times \text{Total EH volume}(0.025\text{L}) \times 0.88}{\text{Total theoretical xylan (g) in initial biomass}} \times 100 \tag{2}$$

3. Results and discussion

3.1. Least cost formulation to identify geographical location for a bio-refinery

By using LCF, we selected a geographical location to examine a biomass blend scenario beyond the U.S. Corn Belt and with a recalcitrant feedstock. Such a case study would fully test not only prospective geographical sites but also the application of predictive

model of a bio-refinery in such a site. Fort Myers suburban area of Lee County in Florida, with access to fresh water from the Caloosa-hatchee River and proximity to shipping routes and fuel-consuming urban counties, seemed to be a good fit for this study, see Fig. 1.

The primary source of biomass in this region is EC, a recalcitrant feedstock that is available at 1000 US dry tons per year in 2020, but will grow to 50,000 US dry tons per year in 2030, see Fig. 1(a). A commercial-scale bio-refinery is expected to process approximately 2205 dry U.S. tons of biomass feedstock per day, for 330 days a year (Sadhukhan et al., 2014; Tao et al., 2014). About 500,000 US dry tons of SG per year in 2030 can be included in the feedstock supply chain of a bio-refinery in this region, see Fig. 1(b). With these two feedstocks combined, a bio-refinery can be operated for 250 days a year. In this study, we assumed CS to be a higher quality feedstock and while CS is not available in the immediate geographical area, see Fig. 1(c), transporting the bio-mass and including it in the blends is an option. Including CS in the blends could not only ensure continuous operation of the bio-refinery throughout the year, but also improve the quality of biomass blend (Thompson, 2016). Blending high-quality biomass has to be implemented to the extent that the overall yields of

intermediates and products reach desirable levels. From a logistics point of view, a bio-refinery in Lee County will need up to 540 dry tons of CS per day, which can be provided by nearby counties in the state of Georgia (Searcy et al., 2007). If blending high-quality bio-mass can help improve overall conversion yields, farmers and local government will be incentivized to grow corn crops in Lee country itself.

From a feedstock quality point of view, to determine the minimum concentration of high-quality feedstock required in a biomass blend, it is essential to test and study yield changes that occur when feedstock ratios are varied. Statistical design of experiments and analysis become vital in predicting this effect of blending biomass feedstocks. In this study, we designed and developed a predictive model to identify either the optimal blend ratios within LCF limits, deconstruction conditions, or both to maintain desirable product yields in a bio-refinery in the Lee County.

3.2. Design of experiments and predictive model development

A response variable that will be most representative of the variability in biomass blends was necessary for initiating the experimental design. We selected glucose yield (percentage of
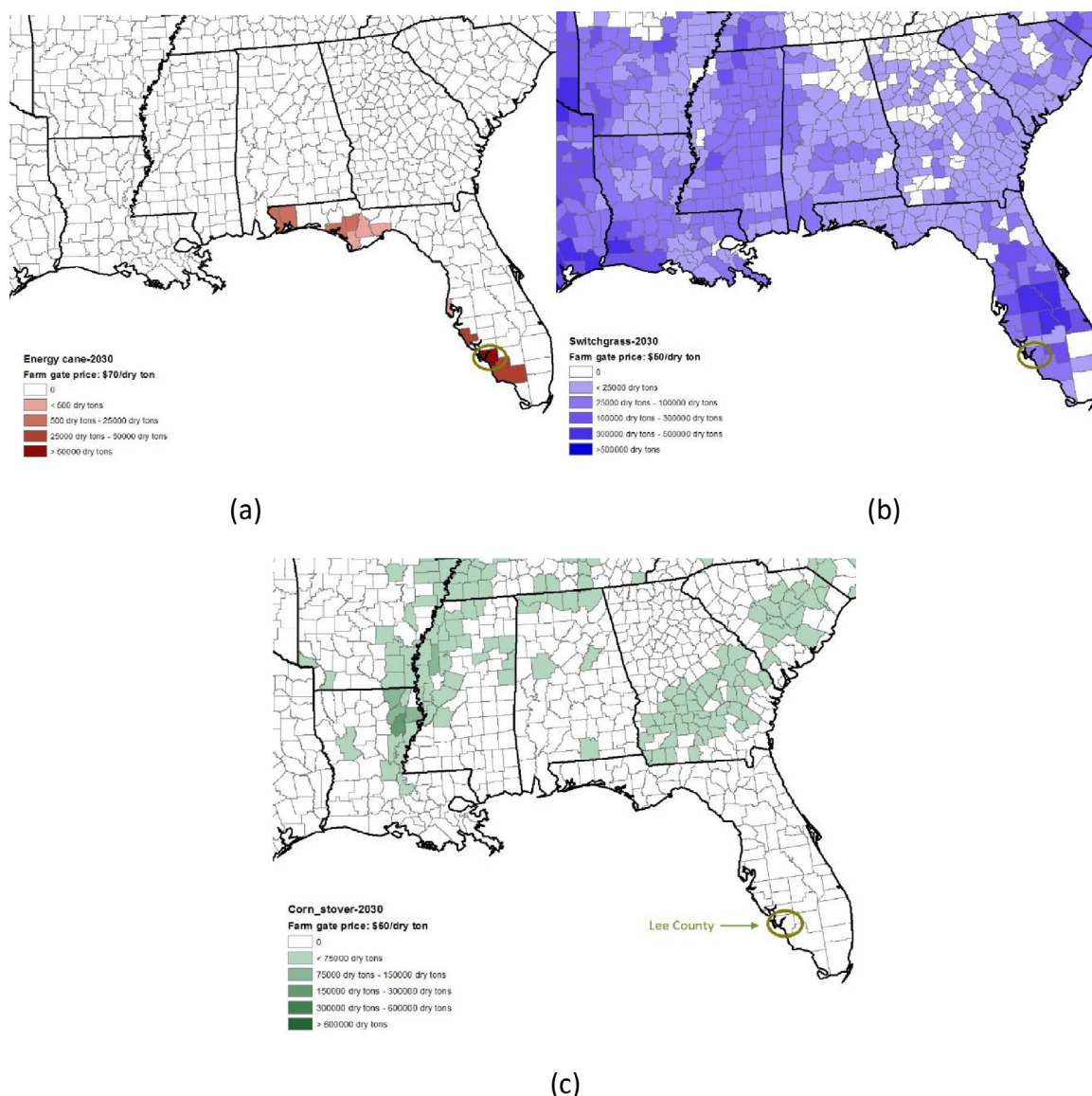

(a)

(b)

(c)

Fig. 1. Expected availability of (a) energy cane, (b) switchgrass, and (c) corn stover in the south-eastern United States in the year 2030.

theoretical maximum) after deconstruction as the response variable in this study to avoid potential inconsistencies that stem from including product yields of downstream processes. Xylose yields and furfural concentrations were also monitored. Literature indicates that glucose yield is highly susceptible to changes in deconstruction process conditions, i.e., chemical pretreatment and enzymatic hydrolysis treatment conditions. To limit the number

of variables in our design, we assumed that enzymatic hydrolysis process has a lower impact than pretreatment variables on glucose yields and performed only a single level of enzymatic treatment, uniformly across all experimental studies. We confirmed the same by conducting a separate pilot study on 24 blends with a higher enzyme loading, see Fig. 2(a) and (b). The average of differences in glucose yields for the 24 blends was less than 10%. In summary, we incorporated (i) blending ratios of the three feedstocks, (ii) pretreatment catalysts, (iii) pretreatment reaction temperatures, and (iv) pretreatment reaction times as key factors of interest and studied their impact on our response variable, glucose yield.

Prior to designing experiments, we performed tests to ascertain the reproducibility of pretreatment and enzymatic hydrolysis studies by conducting triplicate experiments, as given in Table 2. Glucose yield (% of the theoretical) along with standard deviation from these replication studies are also presented in the table. The yield averages were used to baseline the predictions from our model. Most treatments exhibited variability around 5% (of theoretical) in glucose yield, with dilute acid treatments leading to least variability, <2%. It is important to note that this variability represents the error associated with both experimental work and analytical measurements of glucose concentrations.

Experimental design was developed using SAS JMP® (Cary, NC) with a mixed model approach, emphasizing on individual differences that arise from blending the three feedstocks and treating it as a single difference. Biomass blends were allowed to vary from singular feedstocks to a variety of ratios of each of the three feedstocks. Thereby, for a given treatment, the ratio of EC, SG, or CS in the biomass blend could be 1:1:1, 5:2:1, or 1:0:0, etc. As for pretreatment, except pretreatment catalysts that were identified to be categorical variables, all others were designated to be scaled variables. Consequently, as a categorical variable, the design allowed either dilute acid, dilute alkali, or IL for a particular treatment but not the combination of two or three of them. As a single categorical variable with three levels of treatment, pretreatment had two degrees of freedom. Since IL is feedstock agnostic and has been previously shown to deconstruct feedstock blends efficiently, we considered it to be the control for pretreatment variable (Li et al., 2013), i.e. model will report the impact of dilute alkali and dilute acid treatments in comparison with IL pretreatment.

To produce useful effects, after reviewing comprehensive literature evidence, we identified varying ranges of operational parameters for each of the three pretreatment catalysts (Elander et al., 2009; Li et al., 2011, 2010; Lloyd and Wyman, 2005; Wyman et al., 2005a,b). By scaling reaction temperatures and times factors for each pretreatment catalyst, we were able to develop a uniform experimental design in SAS JMP®, which was translated prior to experimentation. As such, 1% temperature for alkali pretreatment
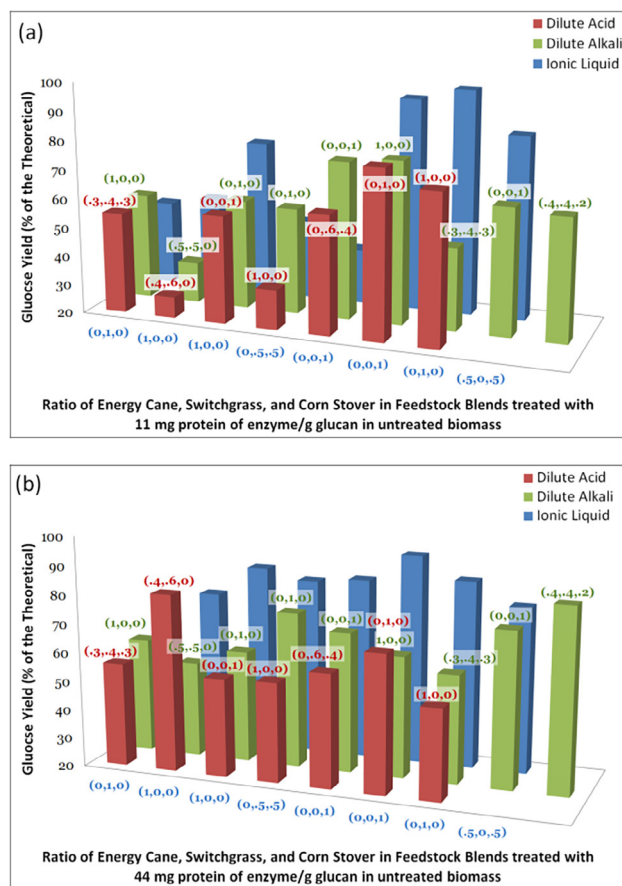

Fig. 2. Glucose yields (% of theoretical) observed in 24 treatments with varying ratios of energy cane, switchgrass, and corn stover in feedstocks tested with dilute acid, dilute alkali, and ionic liquid catalysts at varying pretreatment reaction temperatures and times. (a) Enzymatic hydrolysis was conducted with CTec2® and HTec2® at a loading of 40 and 4 mg protein/g glucan in untreated biomass. (b) Enzymatic hydrolysis was conducted with CTec2® and HTec2® at a loading of 10 and 1 mg protein/g glucan in untreated biomass. Data interpretation with bar chart is limiting.

Table 2
Experimental conditions and glucose yield (% of theoretical) in reproducibility study testing various biomass blends with three pretreatment catalysts.

Treatment number	Block #	Pretreatment	Feedstock ratios			Temperature		Time		Glucose yield[*]
			Energy cane	Switch grass	Corn stover	%	°C	%	Min	Actual
1	1	Dilute acid	0.3	0.4	0.3	1	140	45	30	57.30 ± 0.42
2	1	Dilute acid	0.4	0.4	0.2	1	140	45	30	54.55 ± 1.48
3	1	Dilute acid	0.2	0.2	0.6	1	140	45	30	65.37 ± 1.77
4	2	Dilute alkali	0.3	0.4	0.3	100	120	1	60	57.77 ± 7.03
5	2	Dilute alkali	0.4	0.4	0.2	100	120	1	60	56.55 ± 1.00
6	2	Dilute alkali	0.2	0.2	0.6	100	120	1	60	60.79 ± 8.23
7	3	Ionic liquid	0.3	0.4	0.3	100	160	1	60	86.94 ± 4.71
8	3	Ionic liquid	0.4	0.4	0.2	100	160	1	60	67.51 ± 3.66
9	3	Ionic liquid	0.2	0.2	0.6	100	160	1	60	74.53 ± 5.67

Notes: Pretreatment and enzymatic hydrolysis were performed at solids loading of 10% and 4% w/w (untreated dry biomass in slurry), respectively; enzyme loading of 10 and 1 mg protein/g glucan of CTec2 and HTec2, respectively, in untreated biomass was applied to all pretreated slurries.
[*] Percentage of theoretical maximum as observed in hydrolysate after pretreatment and enzymatic hydrolysis; [i]N.D – Not Detected.

was 55 °C but for acid pretreatment the same was 140 °C. Given below is the range for each of the variables.

- Feedstock compositions (scaled): EC (0–100%), SG (0–100%), and CS (0–100%).
- Pretreatment catalyst (categorical): dilute acid, dilute alkali, or IL.
- Pretreatment reaction temperatures (scaled): dilute acid (1–100%) 140–180 °C, dilute alkali (1–100%) 55–120 °C, IL (1–100%) 120–160 °C.
- Pretreatment reaction times (scaled): dilute acid (1–100%) 5–60 min, dilute alkali (1–100%) 1–24 h, IL (1–100%) 1–3 h.

Per the given ranges, if the experimental design required us to perform a treatment of 100% EC with IL at 1% temperature and 100% time, we treated 1 g of EC with 9 g IL at 120 °C for 180 min (Test #6 in Table 3). Similarly, if we were to perform pretreatment of a biomass blend with 30, 40, and 30% of each of the feedstocks and treat with dilute alkali at 39% temperature and 1% time, we blended 0.3, 0.4, 0.3 g of EC, SG, and CS and treated the biomass blend with 9 g of 1% (w/w) sodium hydroxide at 80 °C for 60 min (Test #16 in Table 3). Reaction temperature was used as the blocking factor, as changing temperature in sand and oil baths during pretreatment experiments was the most time-consuming task and avoiding it was prudent.

Whereas the model was developed to serve the purpose of identifying optimal deconstruction conditions to maximize glucose yields, to build a robust model, it was imperative that we obtain glucose yield data representing the entire 0–100% (theoretical maximum) range. Such data would help avoid distortions in the model and skewed predictions from it. Accordingly, to obtain low and high sugar yields, we added a constraint to limit the sum of

Table 3
Design of experiment for first 24 conditions with varying factors for each treatment along with response variables, glucose and xylose yields and furfural concentrations.

Treatment number	Block #	Pretreatment	Feedstock ratios			Pretreatment temperature		Pretreatment time		Sugar yield[*]		Furfural conc (g/l)
			Energy cane	Switch grass	Corn stover	%	°C	%	min	Glucose	Xylose	
1	1	Ionic liquid	0.0	1.0	0.0	1	120	39	106.8	49.20	30.81	N.D.[I]
2	1	Dilute acid	0.3	0.4	0.3	1	140	100	60	54.75	80.04	0.19
3	1	Dilute Alkali	1.0	0.0	0.0	1	55	100	1440	56.54	89.47	N.D.[I]
4	1	Dilute Alkali	0.5	0.5	0.0	1	55	39	589	33.96	42.72	N.D.[I]
5	1	Dilute Alkali	0.0	1.0	0.0	1	55	100	1440	57.82	55.22	N.D.[I]
6	1	Ionic liquid	1.0	0.0	0.0	1	120	100	180	52.33	52.19	N.D.[I]
7	2	Dilute acid	0.4	0.6	0.0	100	180	1	5	27.21	73.18	N.D.[I]
8	2	Dilute acid	0.0	0.0	1.0	100	180	60	38	57.33	5.46	1.69
9	2	Dilute Alkali	0.0	1.0	0.0	100	120	1	60	56.78	51.24	N.D.[I]
10	2	Dilute Alkali	0.0	0.0	1.0	100	120	1	60	74.60	48.31	N.D.[I]
11	2	Dilute Alkali	1.0	0.0	0.0	100	120	1	60	53.33	0.00	N.D.[I]
12	2	Ionic liquid	1.0	0.0	0.0	100	160	1	60	74.07	73.05	N.D.[I]
13	3	Ionic liquid	0.0	0.5	0.5	39	135	100	180	47.12	25.71	N.D.[I]
14	3	Dilute acid	1.0	0.0	0.0	39	155	1	5	33.81	83.85	N.D.[I]
15	3	Ionic liquid	0.0	0.0	1.0	39	135	1	60	38.98	24.18	N.D.[I]
16	3	Dilute Alkali	0.3	0.4	0.3	39	80	1	60	48.71	51.77	N.D.[I]
17	3	Dilute acid	0.0	0.6	0.4	39	155	1	5	61.27	77.83	N.D.[I]
18	3	Dilute Alkali	0.0	0.0	1.0	39	80	100	1440	64.19	39.18	N.D.[I]
19	4	Dilute acid	0.0	1.0	0.0	80	172	80	49	78.30	22.91	1.35
20	4	Ionic liquid	0.0	0.0	1.0	80	152	80	156	93.51	23.23	N.D.[I]
21	4	Ionic liquid	0.0	1.0	0.0	80	152	80	156	97.71	24.95	N.D.[I]
22	4	Ionic liquid	0.5	0.0	0.5	80	152	1	60	83.63	62.80	N.D.[I]
23	4	Dilute Alkali	0.4	0.4	0.2	80	107	80	1159	62.68	60.88	N.D.[I]
24	4	Dilute acid	1.0	0.0	0.0	80	172	80	48.8	72.26	36.68	1.6

Notes: Pretreatment and enzymatic hydrolysis were performed at solids loading of 10% and 4% w/w (untreated dry biomass in slurry), respectively; Enzyme loading of 10 and 1 mg protein/g glucan of CTec2 and HTec2, respectively, in untreated biomass was applied to all pretreated slurries.
[*] Percentage of theoretical maximum as observed in hydrolysate after pretreatment and enzymatic hydrolysis.
[I] N.D – Not Detected.

Table 4
ANOVA with parameter estimates for each factor tested to model (a) glucose yields and (b) furfural concentrations.

Factor	Coefficients	Standard error	t-ratio	Prob > [t]
(a)				
Energy cane	62.09	3.64	17.02	<0.0001
Switchgrass	68.08	3.85	17.65	<0.0001
Corn stover	73.22	3.79	19.31	<0.0001
Pretreatment [Alkali]	-9.98	2.72	-3.66	0.0005
Pretreatment [Acid]	−5.81	2.80	−2.08	0.0417
Temperature (1, 100)	3.46	2.56	1.35	0.1827
Time (1, 100)	3.03	2.87	1.05	0.2961
(b)				
Energy cane	0.26	0.09	2.89	0.0052
Switchgrass	0.27	0.10	2.82	0.0064
Corn stover	0.24	0.09	2.59	0.0117
Pretreatment [Alkali]	−0.25	0.07	−3.65	0.0005
Pretreatment [Acid]	0.51	0.07	7.37	<0.0001
Temperature (1, 100)	0.24	0.06	3.86	0.0003
Time (1, 100)	0.18	0.07	2.52	0.0142

scaled pretreatment reaction time and temperature to less than 160%. When pretreatment reaction temperature was raised to a 100% of the scale, reaction time could not be raised beyond 60% and vice versa. Similarly, we also set a lower limit of 40% for a difference between the two scaled parameters, i.e. reaction time was not allowed to be set below 60% when reaction temperature was set at 100%, etc. These restrictions allowed us to hone the design to avoid results from several extreme pretreatment condition scenarios and potentially provide an entire gamut of glucose yield numbers.
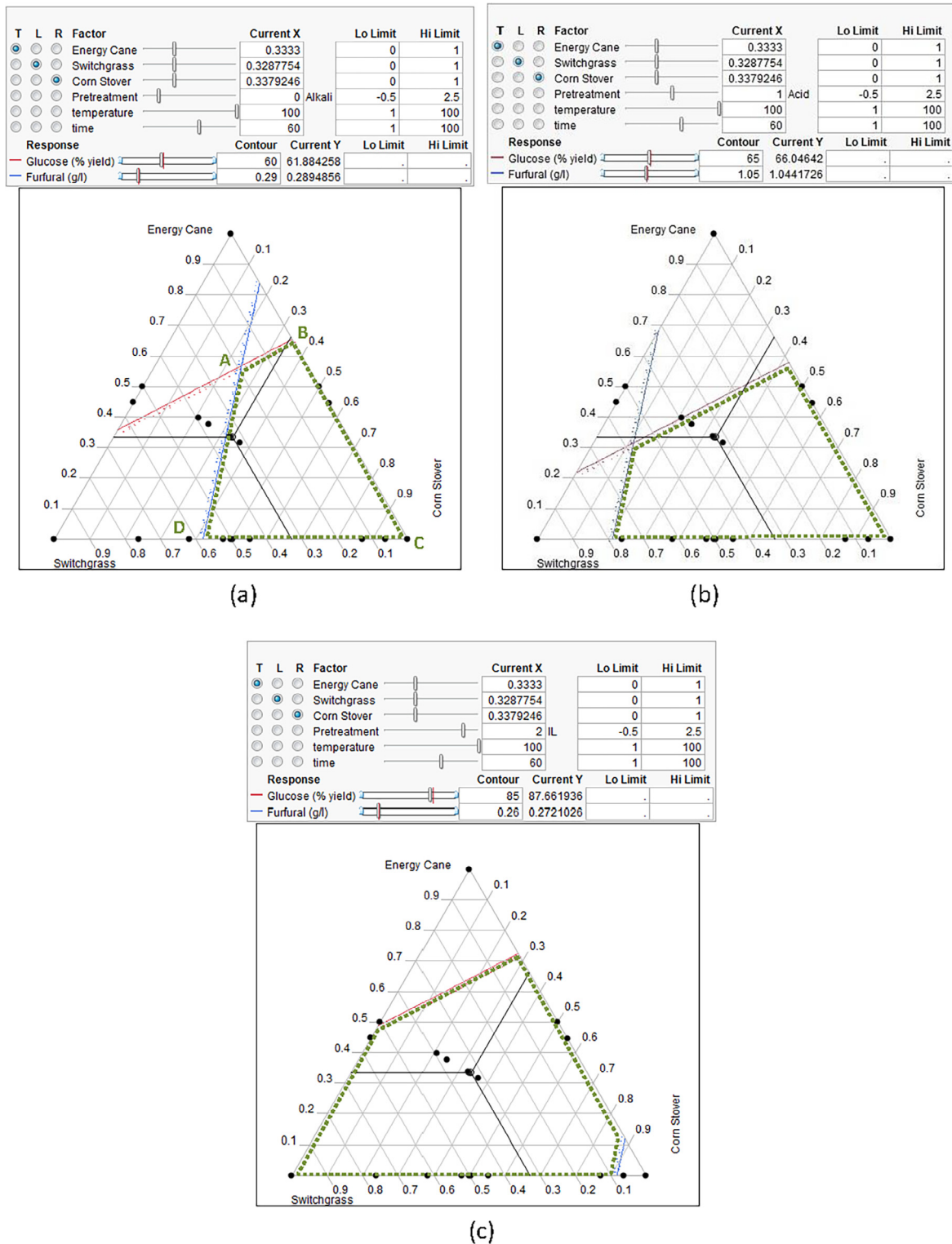
Fig. 3. Ternary plots indicating an envelope of predicted feedstock blend ratios that can enable predetermined theoretical glucose yields of (a) 60% (of theoretical) through dilute alkali, (b) 70% (of theoretical) through dilute acid, and (c) 90% (of theoretical) through ionic liquid pretreatments followed by enzymatic hydrolysis with CTec2® and HTec2® loading at 10 and 1 mg protein/g glucan in untreated biomass blends. Envelope is presented as the area enclosed in the green trapezoid in the (a) ternary plot.

3.3. Glucose yield data analysis and visualization

To develop a linear mixed effect model with glucose yields, we performed analysis of the data with Restricted Maximum Likelihood, or REML, method that was available through SAS JMP®. REML was selected in place of the traditional Expected Mean Squares as this method is appropriate with unbalanced data in our mixed model (e.g., repetition of a particular biomass blend in the experimental design) (SAS, 2017). Per the model, reaction temperature and time had no significant impact on glucose yield, with p-values of 0.31 and 0.25, respectively, as shown in Table 4(a). This result was unexpected, as previous literature has shown that both reaction time and temperature substantially impact glucose yields (Lloyd and Wyman, 2003, 2005). However, we chose a range of reaction temperatures and times that have previously shown to impact the glucose yields and further constrained the upper and lower limits of the sum of scaled pretreatment times and temperatures to 160 and 40%. As such, within this optimized range of combinations of reactions temperature and times, it is possible that these parameters did not have a statistically significant impact. The ratios of the three feedstocks had a significant impact on glucose yields (p-value < 0.0001). Alkali and acid pretreatments had lower level impacts on glucose yields (p-values at 0.0005 and 0.0417, respectively) but were statistically significant, when analyzed against IL pretreatment.

The p-value from lack of fit tests for glucose and furfural were 0.68 and 0.70, indicating that there is no evidence of the model not fitting the data well. From these results, we can conclude that glucose yield was most dependent on feedstock ratios over all other factors tested and alkali pretreatment was able to generate most impact on glucose yield. Washing of IL pretreated solids prior to enzymatic hydrolysis removed much of the hydrolyzed xylan in the washes. As such, we replaced xylose yields with furfural concentration in the model, as the lack of furfural is a good indicator of the quality of sugars for downstream fermentation. Interestingly, all factors tested in this study had a significant effect on furfural concentrations, but as expected acid pretreatment and reaction temperature had the most impact, see Table 4(b).

Researchers often use a bar or line chart to present glucose yield data (Elander et al., 2009; Li et al., 2011, 2010; Lloyd and Wyman, 2005; Wyman et al., 2005a,b). However, due to the six factors that include three feedstocks and three deconstruction-related factors, bar charts were limited in their ability to display our data, see Fig. 2(a) and (b). While we were able to plot glucose yield as a function of the three pretreatment catalysts, the varying feedstock ratios and reaction temperatures and time were not represented linearly on this chart type. Any further labeling of individual treatments would only make the graph more obscure. Furthermore, the average of drop in glucose yield due to reduced enzyme loading, was at 9.92 ± 21.27%. The very high standard deviation indicates that the impact of feedstock blends is much higher than can be explained by averages or bar charts. As such, we relied on SAS JMP® not only for data analysis, but also visualization of the results and interaction with the model. A ternary plot mixture profiler with the three feedstocks represented by the three corners was used to present glucose and furfural predictions. Since the model was also generated in JMP®, the profiler was able to provide us the opportunity to interact with the model, i.e., all factors were provided on a sliding bar allowing the user to vary these factors and view the changes in predictions in real time, see Fig. 3(a).

Dilute alkali, dilute acid, and IL were presented at levels 0, 1, and 2 of the pretreatment catalyst factor, even though the levels themselves do not represent an increase or decrease in the intensity of pretreatments. The trapezoidal area, given in green, enclosed by glucose yield and furfural concentration curves, given in red and blue respectively, represents all blend ratios that can generate the predetermined glucose yield of 60% (of theoretical) with furfural concentrations at a level lower than the set level of 0.29 g/L.

To achieve 60% (of theoretical) glucose yield with dilute alkali pretreatment, we require a CS ratio of at least 0.26 in every blend; represented as corner A in the green trapezoid in Fig. 3 (a). We can completely avoid SG by switching over to corner B, but only with at least 0.35 CS in such a blend. Video 1 (https://youtu.be/fhaTfZbCsEM) is a video capture of the interactive JMP visualization tool of our model. We can also eliminate EC from the blend but with at least 0.42 CS included in the biomass blends; represented as corner D. Corner C is the control test of singular CS feedstock. Fig. 3(b) depicts the blend envelope with acid pretreatment and, as expected, the high xylan content of SG increased furfural concentrations and reduced its prospects as a blend component. When we switch to IL without changing any other parameters, as in Fig. 3(c), predetermined glucose yield can be raised up to 85% (of theoretical). In this system, we have much larger envelope that will allow switchgrass ratio as low as 0.04 but only when coupled with at least 0.27 CS ratio. We can use this tool to change pretreatment reaction temperature and time and instantly predict the impact of these changes on feedstock envelopes and glucose yields. The interactive feature of this visualization is essential in the application of this model as we anticipate bio-refineries, much like petroleum refineries, will be required to quickly adapt to feedstock variability. The continuous envelopes of feedstock blends generated by this model for a given situation can help sugar and fuel producers conveniently and rapidly vary feedstock compositions to ensure a constant supply of feedstock input or reduce feedstock costs, while maintaining or improving overall glucose yields. Since, the model presented in this study is based on blends of EC, SG, and CS, the predictions are applicable only to the various blends of these three feedstocks. A separate geographical location, with a new set of feedstocks, will require a model to be generated based on the glucose yields from the deconstruction studies conducted on a few combinations of those particular feedstocks.
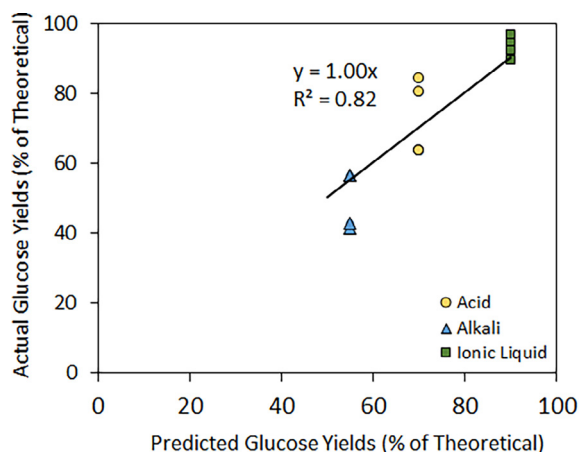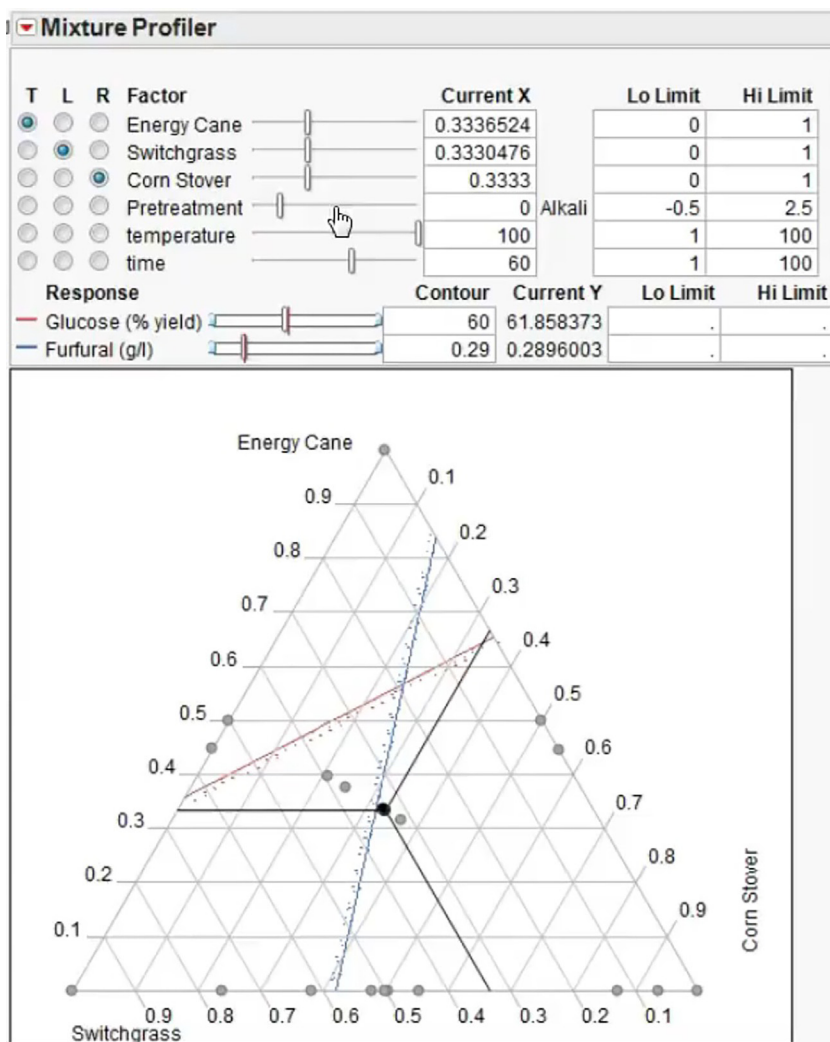

Fig. 4. Results from validation experiments; Actual vs. Predicted.

Video 1. JMP Predictive Model Interactive Visualization (https://youtu.be/fhaTfZbCsEM).

3.4. Predictive model validation

The model presented in this study was validated by performing 13 independent deconstruction tests that were not in repetition to any of the tests conducted for model development. The correlation between actual and predicted glucose values, presented in Fig. 4, established the model and validated the predictions. But this graph is meaningful if we had only one explanatory variable, unlike our model where there were multiple variables to consider. Hence, marginal residuals from the model were used to reflect prediction error based only on the fit of main effects. Residuals plots, glucose yield residuals plotted against the predicted glucose yields, were studied to understand the effect of feedstock ratios and reaction conditions. Positive values for the residuals (on the y-axis) indicate that the prediction was too low and vice versa, making zero value the desirable prediction.

Typically, residual values are expected to be scattered randomly around zero. In this study, some of the data points were clustered around the higher and lower values, indicating that the predictions were not well developed. These data points represented glucose yields from IL pretreatment. Due to the typically high glucose yields observed from IL pretreatment, irrespective of choice of feedstock ratio and reaction temperature and time, the model was unable to avoid inflating glucose yield predictions from IL. While IL is an excellent catalyst to breakdown biomass blends, modeling glucose yields from this pretreatment was challenging.

Glucose yields from dilute acid and alkali pretreatments varied throughout the range making the predictions for these catalysts valid.

The predictions from a validated model can be deemed useful only when applied in a real-world scenario. In a follow up to this study, we further tested the application of this model by testing some of its predictions at a higher solid loading of 30% (w/w) during pretreatment. We also tested the quality of sugars released from these higher solids loading deconstruction by fermenting the hydrolysates to ethanol. The results from the higher solid loading study were applied to a techno-economic analysis model to further validate the application of predictive modeling for biomass blending in commercial-scale bio-based manufacturing.

4. Conclusions

Predictive modeling was used to identify optimal biomass blends that will allow the blending of low-quality, low-price feedstock with higher quality ones to not only expand the scope of establishing a bio-refinery in geographical areas beyond the Corn Belt but also reduce costs. Through the predictive model, we also identified optimal deconstruction conditions to produce at least 60% (of theoretical) glucose yields from various blends, by varying pretreatment catalysts and reaction temperature and time. In a separate publication, we elucidated the application of model predictions in commercially relevant studies, such as high-solid

loading deconstruction and fermentation, and techno-economic analysis.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.06.156.

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