Title

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Publication Date

2019

DOI

10.1016/j.biortech.2018.09.103

Peer reviewed

Simultaneous application of predictive model and least cost formulation can substantially benefit biorefineries outside Corn Belt in United States: A case study in Florida

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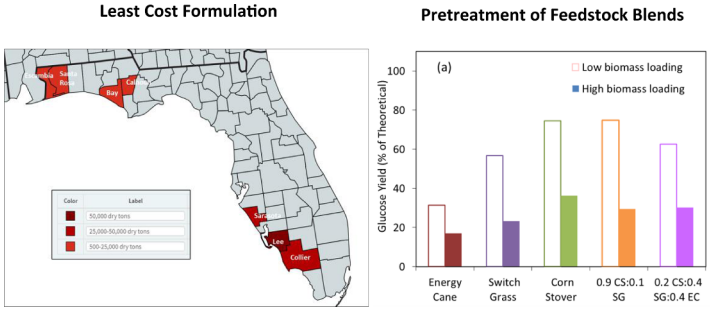
GRAPHICAL ABSTRACT



ABSTRACT

Previously, a predictive model was developed to identify optimal blends of expensive high-quality and cheaper low-quality feedstocks for a given geographical location that can deliver high sugar yields. In this study, the optimal process conditions were tested for application at commercially-relevant higher biomass loadings. We observed lower sugar yields but 100% conversion to ethanol from a blend that contained only 20% high-quality feedstock. The impact of applying this predictive model simultaneously with least cost formulation model for a biorefinery location outside of the US Corn Belt in Lee County, Florida was investigated. A blend ratio of 0.30 EC, 0.45 SG, and 0.25 CS in Lee County was necessary to produce sugars at high yields and ethanol at a capacity of 50 MMGY. This work demonstrates utility in applying predictive model and LCF to reduce feedstock costs and supply chain risks while optimizing for product yields.

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1. Introduction

Bio-based manufacturing in the United States, thus far, has relied heavily on optimizing processing conditions for a single feedstock source, such as corn stover. This approach limits establishing biorefineries in geographical areas that have access to substantial lignocellulosic biomass, but from varying feedstock sources. For example, more than 1 million dry tons of orchard and vineyard prunings were available in Florida for biofuel production in 2016 (Langholtz et al., 2016). INEOS Bio was the first commercial-scale cellulosic ethanol plant established in 2013 to convert Florida's different sources of feedstocks including vegetative wastes, agricultural wastes, and municipal solid wastes (Jose and Bhaskar, 2015; Lubowski et al., 2002). The Idaho National Laboratory (INL) has been developing a Least Cost Formulation (LCF) model to identify geographical areas across the United States that present such increased availability of low-cost feedstocks and thereby opportunities of biomass blending (Ray et al., 2017; Sun et al., 2015; Williams et al., 2016). Researchers at the Advanced Biofuels Process Development Unit (ABPDU), Lawrence Berkeley National Laboratory (LBNL) further investigated recommendations from LCF, but from a bio-processing point-of-view, by optimizing feedstock ratios in blends and the associated conversion conditions to achieve high yields and/or lower costs. A collaboration among researchers from ABPDU, INL, and Sandia National Laboratories (SNL) helped develop a predictive model that could rapidly identify optimal feedstock ratios in tandem with optimal deconstruction parameters in a given geographic area (Narani et al., 2017).

The geographical area chosen for this predictive model development was Lee County in Florida, primarily because it is situated further away from the Corn Belt and has access to an abundant but recalcitrant feedstock, energy cane (EC). Per LCF, switchgrass (SG) was also abundantly available in the region and could be easily incorporated into a blend. Corn stover (CS) was chosen to also be a part of the blends as it was assumed it to be representative of a high-quality feedstock in this study. The singular feedstocks and biomass blends were pretreated with either dilute acid or dilute alkali or ionic liquid (IL) followed by enzymatic hydrolysis and then measured for sugar yields. When pretreated with dilute alkali, we observed that a 1:9 biomass blend of EC and CS led to glucose yield of 71.22% (of theoretical). This yield was similar to that from CS alone, at 74.6% (of theoretical), making the blend more preferable in lowering feedstock costs for lignocellulosic sugar production in a biorefinery (Narani et al., 2017). Similarly, a 0.4: 0.4: 0.2 blend of EC, SG, and CS led to a sugar yield of 62% (of theoretical), higher than those observed from EC or SG alone, at 31.46% and 56.78% (of theoretical). Based on these and other results, we developed a predictive model and presented it through an interactive ternary chart that enabled rapid and simultaneous optimization of biomass blends and associated pretreatment conditions. The model itself was validated by independent studies. The ability to instantaneously access predictions from a valid model that can substantially reduce biomass costs also reduces supply chain risks for a biorefinery. The petroleum industry has long been utilizing such models to be able to promptly tune their processing parameters per feedstock variability (Hsu and Robinson, 2007; Hu et al., 2002).

This predictive model was generated from deconstruction studies conducted only at a low biomass loading (LBL); 10% (w/w) dry untreated biomass in slurry during pretreatment and approximately 4% (w/w) dry untreated biomass in hydrolysis slurry. Many lab-scale deconstruction studies are conducted at LBL (Li et al., 2010; Lloyd and Wyman, 2005; Uppugundla et al., 2014; Wyman et al., 2005a; Wyman et al., 2005b), but for this model to be useful in real-world scenarios, it is necessary that the model's predictions are applicable in commercial scale setting for bio-based manufacturing (Li et al., 2013; Sadhukhan et al., 2014; Tao et al., 2014). High biomass loading (HBL) of 30% (w/w) during pretreatment and 20% (w/w) enzymatic hydrolysis are commonly applied in commercial scale bio-based manufacturing (Humbird et al., 2011). Lower water concentration and consequently reduced heat capacity and reactor volume requirements coupled with higher sugar concentrations in hydrolysates are necessary for economical operation of a biorefinery (Humbird et al., 2011). To ensure that this model is applicable in real-world scenarios, in this study, some of the model's predictions were tested at higher biomass loading (HBL) of 30% (w/w) during pretreatment and 12% (w/w) enzymatic hydrolysis. Further, the quality of sugars in these HBL hydrolysates was tested through fermentation to ethanol.

The 0.4:0.4:0.2 EC, SG, and CS blend, comprised mostly of local feedstocks – EC and SG, will have lowered feedstock transportation costs. These lower upfront costs could possibly negate the lower sugar yield of 62% (of theoretical) from this blend, compared to 74.6% (of theoretical) from CS. To investigate this possibility, which compares feedstock quality and transportation costs, in this study, the predictions from this model were integrated with those from LCF by performing an impact analysis. Without such an analysis, we are unable to determine the value of employing the models. This manuscript also briefly probes process economic implications of these models by simulating the results from downstream fermentation studies into a techno-economic analysis (TEA) model. Performing HBL deconstruction studies, testing hydrolysates in fermentations, integrating LCF and predictive model, and performing TEA was necessary to establish a robust modeling platform for commercial-scale bio-based manufacturing.

2. Materials and methods

2.1. Feedstocks and high solid loading pretreatment

Information on feedstocks used in this study was provided in Narani et al. (2017). Experimental details associated with LBL pretreatment and enzymatic hydrolysis conducted for model development were also provided in Narani et al. (2017). The 10 best sugar yielding feedstock and treatment combinations observed during model development, five each with dilute alkali and IL pretreatment catalysts, were applied for HBL pretreatments and enzymatic hydrolysis; treatment conditions and biomass blends are listed in Table 1. Reaction temperatures were scaled for each pretreatment: Dilute alkali (1–100%) 55–120 °C, IL (1–100%) 120–160 °C. as per Narani et al. (2017). Similarly, reaction times were also scaled: Dilute alkali (1–100%) 1–24 h, IL (1–100%) 1–3 h (Narani et al., 2017).

HBL was administered at 30% (w/w) untreated dry biomass in slurry during pretreatment. HBL alkali slurries were prepared by mixing, in 250 mL Pyrex Erlenmeyer flasks, 30 g of dry biomass with 70 g of water containing 1% (w/w) sodium hydroxide. All pretreatments were conducted by placing the flasks either in an autoclave (Primus Sterilizer, Omaha NE, Model# PSS5-G.1-MSSD) to reach 120 °C or a convection oven for the two other reaction temperatures of 65 and 107 °C (Binder, Bohemia, NY). Enzymatic hydrolysis was then performed on alkali pretreated residual solids without any washing, but diluted to 2.5× on mass basis, thereby making solids loading in the hydrolysis step equivalent to 12% (w/w) untreated dry biomass. The hydrolysis procedure and the ratio of other reagents were the same as described in Narani et al. (2017), except hydrolysis was conducted in larger 250 mL Erlenmeyer flasks. An enzyme loading of 11 mg protein/g glucan in untreated biomass, same as in Narani et al. (2017), was administered.

IL biomass slurries at 30% (w/w) were prepared by mixing 30 g of dry biomass with 70 g of 1-ethyl-3-methylimidazolium acetate (EmimAcetate or [C2mim][oAc]) in pure form in a 500 mL Globe reactor (Syrris, UK). The reactor was stirred at 200 rpm with overhead anchor impeller that held a shaft with blades made of Polytetrafluoroethylene. Once the slurry appeared homogeneous, oil from an oil bath was circulated in the reactor jacket to maintain the slurry at desired pretreatment reaction temperature. Julabo temperature control unit (Allentown, PA) was used to regulate the temperature

Table 1
High Biomass Loading deconstruction experiments with dilute alkali and ionic liquids. Pretreatment and Enzymatic Hydrolysis were performed at solids loading of 30% and 12% w/w (untreated dry biomass in slurry); Enzyme loading of 10 and 1 mg protein/g glucan of CTec2 and HTec2, respectively, in untreated biomass was applied to all pretreated slurries.

Treatment Number*	Block #	Pretreatment	Feedstock Ratios			Temperature		Time		Sugar Yield†	
			Energy Cane	Switch Grass	Corn Stover	%	°C	%	min	Glucose	Xylose
9	2	Dilute Alkali	0.0	1.0	0.0	100	120	1	60	23.23	14.47
47	2	Dilute Alkali	1.0	0.0	0.0	100	120	1	60	17.01	15.13
10	2	Dilute Alkali	0.0	0.0	1.0	100	120	1	60	36.27	17.88
72	4	Dilute Alkali	0.0	0.1	0.9	15.6	65.4	25	396	29.42	18.42
23	4	Dilute Alkali	0.4	0.4	0.2	80	107	80	1159	30.26	24.43
33	1	Ionic Liquid	1.0	0.0	0.0	1	120	100	180	65.90	77.06
38	2	Ionic Liquid	0.0	1.0	0.0	100	160	60	132	100	48.80
39	2	Ionic Liquid	0.3	0.3	0.3	100	160	60	132	100	47.66
56	3	Ionic Liquid	0.5	0.0	0.5	100	160	1	60	100	73.30
20	4	Ionic Liquid	0.0	0.0	1.0	80	152	80	156	100	66.89

† Percentage of theoretical maximum as observed in hydrolysate after pretreatment and enzymatic hydrolysis.
* Treatment numbers correspond to those listed in Narani et al. (2017).

of the oil bath. After IL pretreatment was complete, slurry was recovered into 4L Erlenmeyer flask, to which 1.4L of de-ionized water at 70 °C was added for washing. The IL pretreated biomass turned into firm and unyielding solid lumps after washing. The firm slurry lumps were homogenized for 5 min at a high setting (about 20,800 rpm) with a Laboratory blender (LBC 15, Waring Laboratory, Torrington, CT) to obtain uniform dispersion of biomass particles. Since 1.4L of water was added to 100 mL of the pretreated slurry that contained 30 g of feedstock before treatment, solid loading during homogenization was approximately 6.7%. The homogenized slurry was then filtered through a bleached cotton fiber cheese cloth at room temperature to separate pretreated biomass residue from IL-rich liquid phase. An additional 1.5L of DI water was used to thoroughly wash the recovered pretreated biomass to remove residual IL and low-molecular weight lignin that might have been released during pretreatment and may inhibit enzymatic hydrolysis. Homogenized and washed IL pretreated solids with moisture content of 74–80% (w/w), varying per feedstock blends were diluted to achieve HBL at 12% (w/w) solids and were subjected to enzymatic hydrolysis. Pretreated biomass from alkali and IL pretreatments were hydrolyzed with Ctec2 and Htec2 (Novozymes, Davis, CA) enzyme loading at 10 and 1 mg protein/g glucan in untreated feedstock blends, respectively (Narani et al., 2017). The glucose and xylose yields after enzymatic hydrolysis were calculated as per Narani et al., 2017.

2.2. Fermentation and HPLC analysis

The slurries from HBL enzymatic hydrolysis were centrifuged for 10 min at 3220 RCF (or 4000 RPM) in an Eppendorf 5810R centrifuge (Hamburg, Germany). The supernatant, or recovered hydrolysate, approximately 50 mL, was transferred to a 250 mL Pyrex Erlenmeyer flask and autoclaved for 20 min at 121 °C. Three stock solutions: 100 g/L stock solution of yeast extract (BD, Franklin Lakes, NJ), 200 g/L bacto peptone (BD, Franklin Lakes, NJ), and 700 g/L glucose (Sigma Aldrich, MO) were prepared and autoclaved separately. Each experimental flask was then batched to final yeast extract and bacto peptone concentrations of 10 g/L and 20 g/L, respectively. Standard glucose was added only to the control flasks without any hydrolysate. *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (ATCC® 200062™) seed train, seed 1 and seed 2, were generated with the same media as experimental flasks, except with standard glucose instead of the hydrolysates as the carbon source. Seed 1 was inoculated with 0.5% (v/v) of a thawed −80 °C glycerol stock and Seed 2 was inoculated with 2.5 (v/v) of seed 1, respectively. Both seeds were generated in duplicate flasks, where each replicate was prepared to a 70 and 100 mL volume in 250 and 500 mL Erlenmeyer flasks. All flasks were incubated in a Thermo Scientific shaker (Waltham, MA) at 30 °C and 250 RPM for 24 h and 30 h for seed 1 and seed 2, respectively.

Experimental flasks were inoculated with a 5% (v/v) seed 2 culture with OD (600 nm) of 6.0–6.5. Samples were taken periodically at time intervals of 0, 2, 4, 6, 8, 12, 24, and 48, hours and analyzed for glucose and ethanol concentrations on a Dionex HPLC (Thermo Scientific, Waltham, MA) with an Aminex HPX-87H® column with appropriate guard column (7.8 × 300 mm) (Bio-Rad, Hercules, CA). The parameters for HPLC analysis were: (i) column temperature at 65 °C; (ii) detection RI temperature at 50 °C, and (iii) a 5 mM sulfuric acid eluent with a flow rate of 0.6 mL/min for 50 min. Integration and analysis of samples were performed using Dionex Chromeleon® software and identification of monomeric sugars and inhibitors and their individual concentrations were determined relative to known standards listed in Narani et al. (2017). Glucose and ethanol concentrations from the nutrient study were also monitored on Thermo Scientific Gallery (Waltham, MA). We also conducted a shake-flask study on the hydrolysates with varying levels of nutrient dosage. For controls in this nutrient dosage study, glucose was fermented with and without nutrients, yeast extract and bacto-peptone. Ethanol yield (% of the theoretical) for all the time points was determined by the following stoichiometric equations:

$$\frac{Measured\ \ ethanol\ \ concentration\left(\frac{g}{L}\right)}{Measured\ \ initial\ \ glucose\ \ concentration\left(\frac{g}{L}\right)\times 0.51}\times 100$$

2.3. Impact and techno-economic analysis

To understand the impact of various blending scenarios for a biorefinery in Lee county, a detailed analysis was performed to evaluate the impact of simultaneous application of LCF and predictive model. This analysis was conducted under a set of assumptions that can be categorized broadly into three areas: (A) Biorefinery-based, (B) Feedstock-based, and (C) Transportation-based assumptions. These assumptions are listed in Section 3.3 along with the results of our analysis including feedstock, transportation costs and their impact on the economic performance of the biorefinery.

An integrated bio-refinery model was built in SuperPro Designer® (Scotch Plains, NJ), commercially available process simulation software. The bio-refinery configuration, see Fig. 1, was based on the process design proposed by National Renewable Energy Laboratory's (NREL) design report and, except the pretreatment section, all sections were maintained unaltered (Humbird et al., 2011; Konda et al., 2014); see Table 2 for a list of parameters. The pretreatment section in this analysis is based on the experimental protocol designed for IL as pretreatment catalyst and included washing procedure prior to enzymatic hydrolysis. However, for IL pretreatment to be economically successful, it is necessary that we recycle the IL back to pretreatment. As such, this

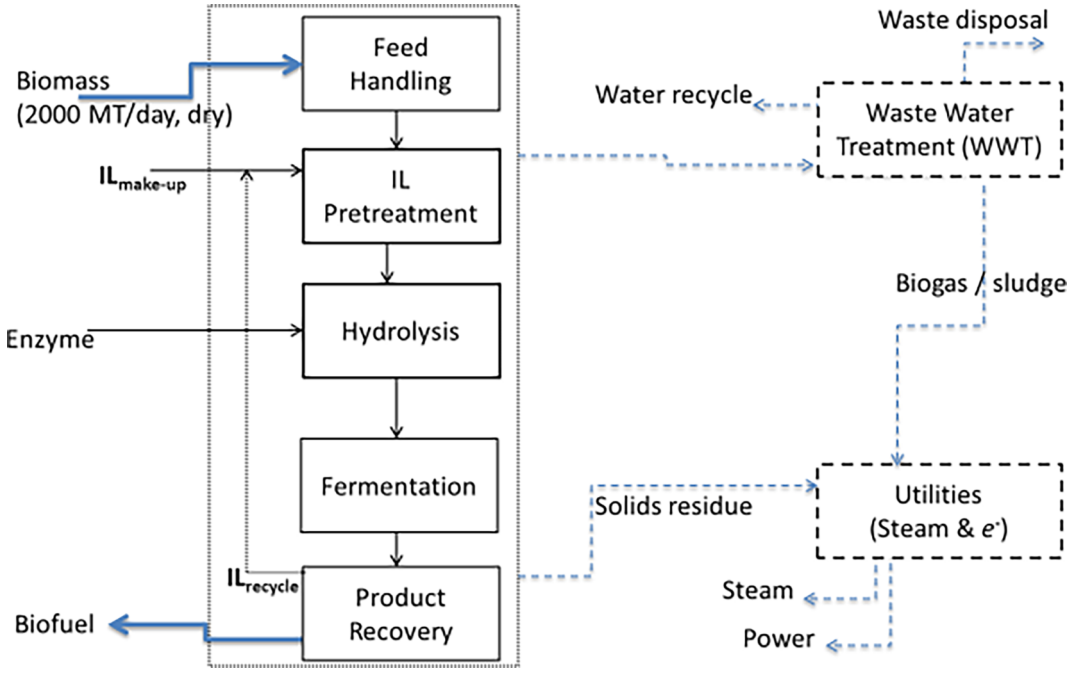


Fig. 1. Simplified block flow diagram of the bio-refinery process model.

Table 2
Key process specifications for the biorefinery with IL pretreatment.

Biomass processed (MT/day, dry)	2000
Moisture content in the delivered feedstock (w/w)	20%
Pretreatment	
Ionic liquid	$[C_2C_1IM][OAc]$
Solids loading (w/w)	30%
Price of IL ($/kg)	10
Water used in multi-stage washing operation (where x = amount of initial biomass on dry basis)	20 ×[a]
IL recovered into the liquid stream after washing	> 99%
Solids recovered after water-wash	
Glucan recovered	95%[b]
Xylan recovered	76%[b]
Lignin recovered	35%[b]
Enzymatic Hydrolysis	
Solids loading (w/w)	12%
Temperature (C)	50
Operating time (h)	72
Enzyme loading (mg/g of glucan; CTec2:HTech2 = 10:1, w/w)	11
Price of enzyme ($/kg of protein)	5
Co-Fermentation of glucose and xylose	
Glucose conversion to ethanol	95%
Xylose conversion to ethanol	85%

[a] While the water usage for washing IL pretreated solids was fairly high (> 50×) in this study, it is likely that a substantial reduction to 20× can be achieved at industrial scale due to recycling, process optimization, etc.

[b] Since experimental data were not available, representative values were assumed based on recent literature (Uppugundla et al., 2014).

Table 3
Key operational and financial assumptions used in techno economic analysis.

Plant life	30 years
Discount rate	10%
Depreciation method[a]	Straight-line (over 10 year period)
Federal taxes	35%
Financing	40% equity
Loan terms	10-year loan at 8% interest rate
Construction period	3 years
First 12 months' expenditure	8%
Next 12 months' expenditure	60%
Last 12 months' expenditure	32%
Start-up time[b]	6 months
Revenues during start-up	50%
Working capital	5% of fixed capital investment
Year of economic analysis	2015

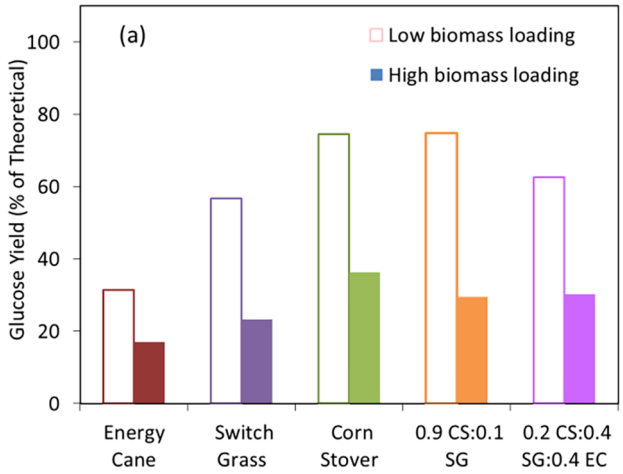
[a] Based on the method available in SuperPro Designer.

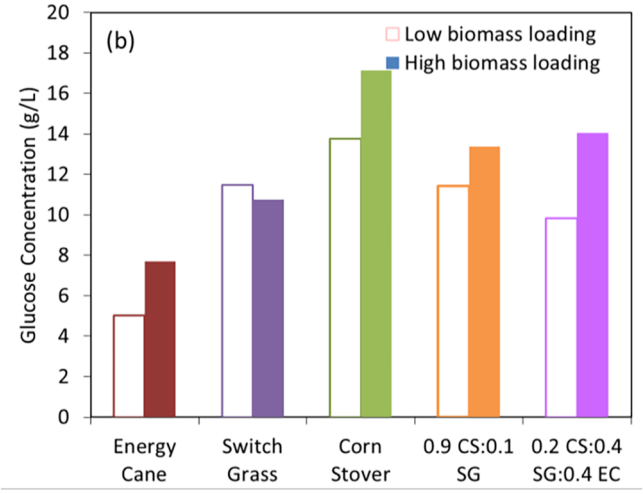
[b] Based on a more recent NREL study (Davis et al., 2013).

simulation also includes a series of recycle process steps on the IL-rich stream.

The economic analysis with the methodology outlined by National Renewable Energy Laboratory (NREL) and key assumptions were given in Table 3 (Davis et al., 2013; Humbird et al., 2011; Uppugundla et al., 2014). In line with NREL's procedure, MESP was used as the metric for economic performance. The IL-rich aqueous stream resulting from the washing process was simulated to be dehydrated through a three-step process that included: (i) 5X concentration using an energy-efficient multi-effect vacuum evaporation section, (ii) ultrafiltration to remove fine residual solids from the concentrated IL-rich liquor, and (iii) pervaporation to separate water from IL, prior to its recycling in moderately dry conditions (< 10% w/w water). Multiple effects (4-effects) were used to improve energy-efficiency and realize a steam economy, or the ratio of dehydrated water to the amount of steam used, of ~2.8. Also, vacuum evaporation unit operation was included to minimize energy penalty by using low pressure steam.

After fermentation of hydrolysate, ethanol from the broth was simulated to be recovered using distillation in the product recovery section. Waste Water Treatment (WWT) section consists of anaerobic and aerobic digesters. Solid residue left after distillation was used as fuel in the co-generation section along with biogas and sludge from WWT section, and external natural gas. Steam and electricity generated from co-generation were used towards the processing needs of the biorefinery. The boiler section was simulated such that the plant was self-sufficient with respect to steam demand while any excess electricity from the on-site multi-stage turbo generator was exported to the grid. Minimum Ethanol Selling Price (MESP) was estimated based on detailed 30-year cash flow analysis corresponding to 10% internal rate of return as the economic performance metric.





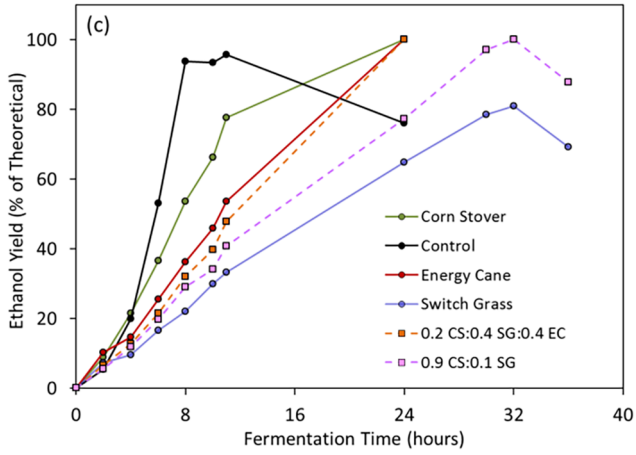
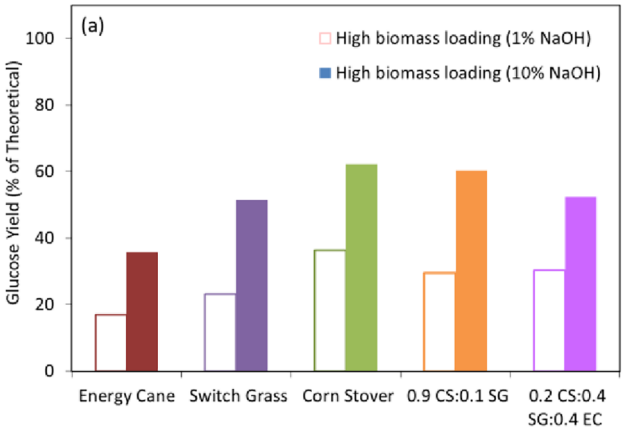


Fig. 2. Glucose (a) yield (% of the theoretical) (b) concentrations (g/L) in hydrolysates after alkali pretreatment and enzymatic hydrolysis of feedstock blends at low and high biomass loading (10 and 30% w/w biomass in slurry) and (c) Ethanol yield (% of theoretical) after fermentation of the sugars in the hydrolysates from high biomass loading pretreatments.

3. Results and discussion

3.1. Application of predictive model in a high biomass loading environment

Sugar yields after enzymatic hydrolysis from HBL alkali pretreatment studies, given in Table 1, were much lower than those for LBL studies, see



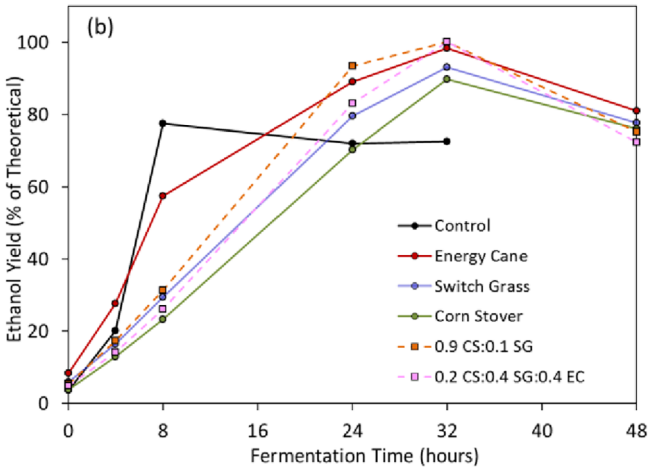


Fig. 3. Glucose (a) yield (% of the theoretical) after alkali pretreatment of feedstock blends with 1% NaOH and 10% NaOH based on 30% w/w biomass loading in slurry and (b) Ethanol yield (% of theoretical) after fermentation of the sugars in the hydrolysates from high biomass and alkali loading pretreatments.

Fig. 2(a). The glucose concentrations were higher in most HBL studies in comparison to those at LBL, due to higher biomass available for conversion in HBL, see Fig. 2(b). The yield drops are primarily due to lack of free water availability during deconstruction in HBL slurries, which caused substantial catalyst mass transfer limitations. These drop in yields occurred irrespective of pretreatment temperature or time, that spanned from 65 to 120 °C and 60 to 1159 min. However, the highest drop in yield occurred in a treatment with least severity, a pretreatment that was administered at 65 °C for 396 min as in Treatment #72 (Narani et al., 2017). In order to apply the predictive model to deconstruction at HBL, it was essential to adopt higher severities through higher alkali loadings. The higher alkali loading (10% w/w based on the dry biomass) led to 1.9 times higher glucose yield in comparison to low alkali loading (1% w/w based on dry biomass) for both singular and mixed feedstocks, see Fig. 3(a).

Sugar yields of IL pretreated EC at HBL followed a similar trend to that of alkali pretreated feedstocks at HBL, i.e. they were lower than the corresponding yields at LBL. However, IL pretreatment of CS, SG, and the blends at HBL led to high sugar yields, similar to those from LBL studies, see Fig. 4(a). IL pretreated hydrolysate, see Fig. 4(a) had 3 times higher glucose yield in comparison to alkali pretreated hydrolysate, see Fig. 2(a). ILs are very effective deconstruction catalysts that can break down many singular or blended feedtsocks (Li et al., 2011; Li

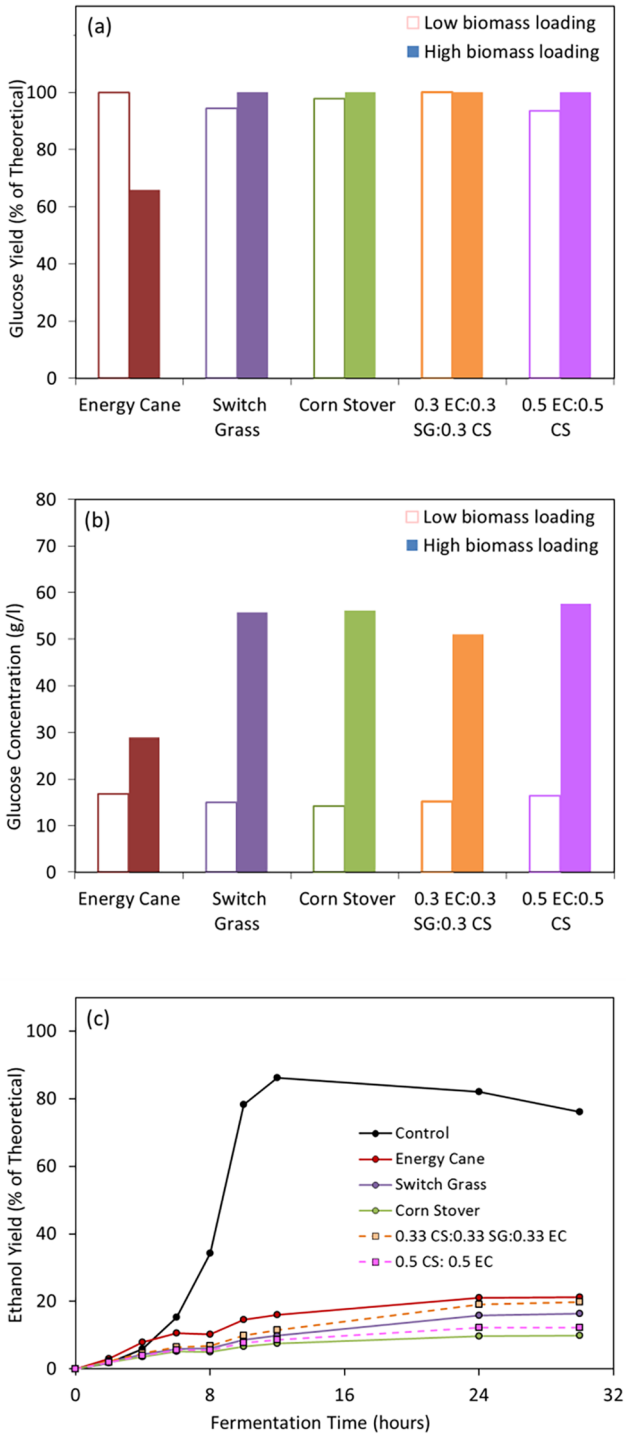


Fig. 4. Glucose (a) yield (% of the theoretical) (b) concentrations (g/L) in hydrolysates after enzymatic hydrolysis of feedstock blends pretreated with ionic liquid (Emim Acetate) at low and high biomass loading (10 and 30% w/w biomass in slurry) and (c) Ethanol yield (% of theoretical) after fermentation of the sugars in the hydrolysates from high biomass loading pretreatments.

et al., 2010; Li et al., 2015; Shi et al., 2013). However, the recalcitrance of EC coupled with mass transfer limitations at HBL led to lower sugar yields than in the case of LBL, reinforcing that EC is highly recalcitrant compared to the more amenable SG and CS and blending EC with high-quality feedstocks is necessary for its use as a feedstock for biomanufacturing.

3.2. Glucose fermentation to ethanol

The ethanol yields (% of theoretical) obtained from fermentation of hydrolysates of HBL alkali deconstruction tests along with control (standard glucose) are depicted in Fig. 2(c). The control samples showed complete conversion of standard glucose into ethanol in just 12 h and ethanol concentrations then followed a downward trend due to consumption by the culture that is devoid of all other carbon sources; see Figs. 2(c), 3(c), 4(c). Glucose in hydrolysates from all alkali pretreated (1% w/w of alkali per dry biomass) feedstocks were converted efficiently with yields ranging from 80 to 100% (of theoretical). SG glucose was not only least fermentable, but also slow in conversion rate with only 65 and 81% (of theoretical) ethanol yields obtained after 24 and 32 h, in comparison to 78 and 100% (of theoretical) ethanol yields generated in 11 and 24 h from CS sugars. It is possible that lignin monomers and oligomers remaining from the unwashed hydrolysate led to the slow fermentation rate of SG hydrolysate. Interestingly, the biomass blend with a CS ratio with at 20% led to 100% ethanol yield (% of theoretical) within 24 h of fermentation, a trend similar to that observed with singular CS. However, the early rate of ethanol production was slower from this 20% CS blend, making it more comparable to singular EC feedstock. Even though we were able to effectively convert most of the sugars derived from HBL alkali pretreatments, the overall process ethanol yields were low, because glucose yields from feedstocks were very low, approximately 17.0–36.2% (of theoretical). In an ideal scenario, we should be able to optimize catalysts and their concentrations to achieve high sugar yields enabling a high overall ethanol yields from feedstock blends at HBL.

We performed deconstruction studies with increased alkali concentration to improve pretreatment efficiency of singular and blended feedstocks. With an increase in alkali loading from 1% to 10% (w/w) dry biomass, ethanol yields of SG sugars improved from 65% (24 h) and 81% (32 h), see Fig. 2(c), to 79% (24 h) and 89% (32 h), as depicted in Fig. 3(b). EC and 0.4 EC blend also generated higher ethanol yields of 98.3% and 100% (of theoretical) at the higher alkali loading. These results indicate that a high performing feedstock, at a ratio as little as 0.2 or 0.4 in a blend with low performing feedstocks can lead to high ethanol yields while reducing feedstock costs.

To further investigate other advantages of utilizing hydrolysates from blended feedstocks, we compared different dosages of nutrients in fermentation. The control flasks with standard glucose as carbon source had final nutrient concentrations of 10 and 20 g/L of yeast extract and bacto peptone, respectively. The two nutrients were progressively reduced to half, one-third, and no nutrient dosages when fermenting CS hydrolysate, i.e. initial yeast extract concentration were at 5, 3.33, and 0 g/L and bacto peptone concentrations were at 10, 6.66, and 0 g/L, respectively. Nutrient dosage level of half in CS hydrolysate studies led to same ethanol yields as those observed from full dosage of nutrients, but only after a longer fermentation time of 30 h, as opposed to 24 h in the full dosage study. As nutrient supplementation was lowered further to one-third of its original dosage, not only was the rate of fermentation reduced, but the final yield from CS hydrolysate was also lowered to approximately 65% (of theoretical). CS hydrolysate without any nutrient dosage led to less than 40% (of theoretical) ethanol yield. Reduced nutrient supplementation comes with a tradeoff, with increased operation time that can negate the financial benefits of lower dosage. This study was further tested with hydrolysates from a blend of equal parts of corn stover and switchgrass (CS/SG) hydrolysate. Whereas, ethanol yields of only 65% (of theoretical) were achieved with half the dosage of nutrients for the blend hydrolysate, about 50% (of theoretical) ethanol yield was observed with one-fourth dosage of nutrients. It is possible that low molecular weight lignin released from SG into the hydrolysate during the deconstruction process is different from that released from CS and is inhibitory towards fermentation.

Based on this study, we conclude that a lower dosage of nutrients either led to a delay in fermentation progress and/or lower fermentation yields.

While sugar yields and concentrations from IL pretreatments were much higher than those from dilute alkali pretreatments, ethanol yields were much lower, see Fig. 4(c). These low ethanol yields were potentially caused by the inhibition from residual IL in hydrolysate on *S. cerevisiae* activity. In the case of IL pretreatment, pretreated solids were washed with water prior to enzymatic hydrolysis, see Section 2.1. In the LBL tests, water mass equivalent to 150 times dry biomass was used (N arani et al., 2017). However, previous TEA studies strongly encourage a reduction in water usage in washing IL pretreated solids (Konda et al., 2014). As such, for the HBL pretreatment studies, we applied water mass equivalent to 80 times the mass of dry untreated biomass. This possibly led to a carry-over of IL into the fermentation process and thereby the reduced ethanol yields. Further washing will most likely lead to a 100% (of theoretical) yield of ethanol (Li et al., 2013). Biocompatible ILs, such as cholinium lysinate, have the potential to eliminate this need for separation step before enzymatic hydrolysis and can improve process economics (Sundstrom et al., 2018).

Both alkali and IL pretreatment processes can be further optimized to improve glucose and ethanol yields at HBL. If a deconstruction and fermentation process was to be optimized together based on LCF and predictive modeling, it is then interesting to gauge the impact of simultaneous biorefinery location selection and feedstock blend optimization. To quantify this impact, we performed an analysis to compare optimal blending scenarios for biorefinery possibilities in Lee county.

3.2.1. Impact analysis

LCF has been developed for the industry to identify geographical locations with adequate lignocellulosic biomass to establish a commercial bio-refinery (Searcy et al., 2014). We developed a predictive model so biorefineries can simultaneously and rapidly choose biomass blend ratios and optimize pretreatment conditions depending on the quantity and quality of each feedstock available at a given time (Narani et al., 2017). In the case study of Lee County, LCF indicated the availability of three biomass feedstocks EC, SG, and CS and, via predictive model, we selected 0.4:0.4:0.2 EC, SG, and CS as the optimal biomass blend. However, to be able to achieve a production capacity of 50 million gallons a year, additional feedstock amounts will have to be transported to Lee county and the associated costs should be optimized (Tao et al., 2016). To calculate the associated transportation costs, we made several assumptions in three categories, as listed below:

A. Biorefinery-based assumptions:

(i) The cellulosic ethanol based biorefinery was assumed to be at production capacity of 50 million gallons/year, similar to literature values of ethanol production from CS and corn grain based feedstocks (Tao et al., 2016).

(ii) The sugar and ethanol yields from the blend ratio (0.4:0.4:0.2 EC, SG, and CS) that led to a maximum glucose and xylose yields of 62.68 and 60.88% (of theoretical) with alkali pretreatment and enzymatic hydrolysis were used to determine biomass needed for biorefinery. The cellulosic glucose from this blend was observed to convert to ethanol at 100% (of theoretical) yield.

(iii) The xylose conversion to ethanol at 85% (of theoretical) yield was used in this analysis (Humbird et al., 2011).

(iv) The amount of EC, SG, or CS required for a biorefinery per year in Lee County was 234,798, 234,798, and 117,399 tons/year.

$$\text{Amount of EC required (tons/year)} = \text{Blend ratio} * \text{Total Biomass required (tons/year)} \quad (1)$$

B. Feedstock-based assumptions:

(i) LCF for the year 2030 depicts feedstock availability for a given county in the format of a range, i.e. with minimum and maximum tons of feedstock available per year. For example, in Collier county, EC available in 2030 can be anywhere between 25,000 and 50,000 dry tons. We averaged the minimum and maximum values to report EC availability as 37,500 dry tons. As such, we calculated the total biomass (EC, SG, or CS) available to a biorefinery in Lee county as a sum of the averages of available feedstock types (tons/year) in Lee and other nearby counties, see Eq. (2).

(ii) Amounts of CS or SG available locally or transported to Lee County were also calculated in a similar fashion, with CS transported from Decatur and Grady counties and Switchgrass from Hendry county; with more SG from Sarasota and Collier counties can be included if more biomass is needed to be supplemented.

$$\begin{aligned}&\text{Total Energy Cane availability (Average) in tons/year}\\&= \text{Sum of Energy Cane availability in Lee County and Other Counties (Availability in Sarosata+ Collier+ Escambia + Santa Rosa+ Bay+Calhoun)}\end{aligned} \quad (2)$$

(iii) The amount of additional biomass of each feedstock type required by the biorefinery but not available in and around Lee county was calculated per Eq. (3).

$$\begin{aligned}&\text{Additional Energy Cane required by a biorefinery in Lee Country}\\&= \text{Energy Cane Available from Eq 2}\\&\quad -\text{Energy Cane Required as per predictive model from Eq 1}\end{aligned} \quad (3)$$

(iv) If the required additional feedstock type, as calculated per Eq. (3), is zero, then there is sufficient biomass of a given feedstock type to operate the biorefinery. If it is positive, then there is excess of a given feedstock type, so the additional biomass from the farthest county was rejected to reduce transportation costs. If the number is negative, then there is shortage of the feedstock type, so biomass from counties further away from Lee county and their respective transportation costs were included in the analysis. When EC was not available, SG and CS were used to supplement the demand by transporting to Lee County by road, rail or ship, depending on the proximity of county.

C. Transportation-related assumptions:

(i) Google Maps was used to calculate the distance to transport a feedstock by road or rail. For transportation by sea, Searoutes.com was used, see Table 4.

(ii) Transportation costs to bring feedstocks from nearby county to Lee

Table 4
Distance from nearby counties to Lee county based on the mode of transportation (rail, road or ship) used for transporting biomass (EC, SG, CS).

County	Distance from Lee County, FL (miles)	
	Road/Rail	Sea
Sarasota, FL	75	N. A.
Collier, FL	100	N. A.
Escambia, FL	N. A.	350
Santa Rosa, FL	N. A.	350
Bay, FL	N. A.	300
Calhoun, FL	N. A.	300
Hendry, FL	70	N. A.
Lee, FL	0	0
Decatur, GA	N. A.	430
Grady, GA	N. A.	400

N. A. – Not applicable, if the mode of transportation was not used in impact analysis.
0 – Distance is zero miles from where biorefinery is located.

county was calculated by using Eq. (4).

$$\text{Transportation cost (\$/ton)} = \text{Direct Fixed cost (\$/ton)} + \text{variable cost (\$/(ton}*\text{mile))} * \text{distance from Lee county (mile)} \quad (4)$$

(iii) Feedstock transportation costs included fixed costs (dependent only on weight) and variable costs (dependent on weight and distance) (Searcy et al., 2007). If feedstocks were transported by truck, fixed cost was set at 4.39 $/ton and variable cost was 0.19 $/(ton. mile). By rail, fixed cost was 14.15 $/ton and variable cost was 0.04 $/(ton.mile). When transported by ship, fixed cost was 34.01 $/ton and variable cost was 0.02 $/(ton.mile). When feedstocks were transported via ship, port charges and transportation costs from shipping port were assumed to be included in the fixed cost.

(iv) EC and SG bales were assumed to be similar to CS bales in weight and volume (1 ton of CS bale was assumed to be same as 1 ton of EC bale and SG bales), so the direct fixed cost (DFC) in a given transportation mode was assumed to be same across feedstocks. The DFC reported for straw/stover category was used for all three feedstocks (Searcy et al., 2007).

(v) Feedstock transportation cost for EC, SG or CS is calculated using Eq. (5).

$$\text{Feedstock Transportation Cost (\$/year)} = \text{Total transportation cost (\$/tons)} * \text{Total biomass available (tons/year)} \quad (5)$$

(vi) Ethanol was assumed to be transported from Lee County to major metropolitan cities in Florida (Miami, Orlando and Tampa) by truck with fixed cost of 3.86 $/ton and variable cost of 0.05 $/ton.mile. The ethanol transportation was also calculated using Eq. (5). Average ethanol transportation cost to major cities from biorefinery was calculated based on the ($/ton of biomass) and not as ($/gallon of ethanol) as the intent was to analyze the impact of LCF and predictive model in reducing total feedstock costs.

Per LCF, the 12,750 tons per year EC is available in each of Escambia, Santa Rosa, Bay, and Calhoun counties that can be procured via ship to complete EC requirement, with a transportation cost of $39.25 per ton or $2.0 MM per year. Further, about 37,500 tons of EC each from Sarasota and Collier Counties is needed to complete EC requirements and available at a transportation cost of $18.9/ton and $23.7/ton respectively, increasing total EC transportation costs to $3.60 MM per year. For SG requirements, about 200,000 tons can be transported from Hendry County at $17.9 per ton that, adding $3.58 MM per year to the operational costs of a biorefinery in Lee County. Other nearby counties: Sarosta and Collier could also be used to supplement more switchgrass input into the biorefinery. Since all CS has to be imported from Grady and Decatur counties in Georgia, the transportation costs via rail to procure the 112,500 tons available from each of the counties will be $29.0 and $30.1 per ton, or a $6.6MM additional transportation costs. The surplus amount of SG (27,702.15 tons/year) and CS (107,601.08 tons/year) was then balanced with additional EC demand (58,797.85 tons/year) that was needed to achieve optimal blend as per predictive model. Hence, to meet the feedstock's demand in Lee County, the blend ratio will have to be adjusted to 0.3 EC, 0.45 SG, and 0.25 CS. A higher SG concentration will have to be accepted by the biorefinery due to transportation costs calculated per the impact analysis. On an average the transportation costs for this biomass blend (0.30 EC, 0.45 SG, and 0.25 CS) was determined to be $19.65 per ton. The adjusted transportation cost in $/year for EC, SG, CS will be $3.6MM, $3.5MM, $4.3MM respectively.

The impact analysis included transportation cost not only to bring feedstocks to Lee County, but also to move ethanol from Lee County to nearby major cities: Miami, Orlando and Tampa at $5.58 per ton of biomass. The average total cost of biomass transportation to Lee County bio-refinery and ethanol transportation from bio-refinery to metropolitan cities was calculated to be $25.23 per ton. In order words, the cost of biomass transported to Lee County will be 27.7 cents per gallon of ethanol produced, approximately 9% when MESP is $3/gallon. Impact analysis coupled with results from LCF and predictive model helped in determining adjusted feedstock blends required to operate a 50 Million gallons/year cellulosic biorefinery outside Corn Belt region (Humbird et al., 2011). In order to understand the process economic implications of these models, detailed TEA was conducted.

3.3. Techno-economic analysis of a bio-refinery based on predictive model

TEA was performed in conjunction with impact analysis (a combination of LCF and predictive modeling) to develop an agile system of modeling to enable rapid decision making on operations that maximize overall profitability of a commercial bio-refinery. For this TEA study, IL pretreatment-based biorefinery case was selected. The reasons for this choice were two-fold. Firstly, the results from impact analysis indicate the need to incorporate a higher concentration of SG, to minimize transportation costs. IL catalysts have shown to generate 100% (of theoretical) glucose yield from SG alone. Secondly, several TEA have already been conducted to estimate the economics of dilute alkali pretreatments-based processes (Stoklosa et al., 2017; Tao and Aden, 2011). This TEA was performed to further our understanding of the economics associated with the novel catalyst, Emim-acetate.

The TEA model in this study represents a mature industrial scale facility (Nth plant) capable of processing 2000 dry MT/day of biomass to produce 50 MM gallons of ethanol. The model is highly integrated and consists of multiple processing areas including pretreatment, hydrolysis, fermentation, product recovery, wastewater treatment (WWT), and cogeneration of steam and electricity. The conversion data, generated through HBL deconstruction studies, detailed in Sections 3.1 and 3.2 were used for this analysis. Three scenarios (S1, S2, and S3), representing deconstruction treatments 70, 15, and 32 in Table 1, were chosen for this analysis. These three scenarios were characteristically different in biomass blend ratios and utilized IL as pretreatment catalyst but at different pretreatment conditions, per predictive model. As shown in Fig. 5(a), all the three scenarios led to similar and near complete glucan conversion, with rather low enzyme loading of 11 mg/g of glucan. While xylose yields in S1 and S3 were high at 94 and 95% (of theoretical) respectively, it was much lower in S2 at only 55% (of theoretical). SG, which was exclusive to the S2 scenario, contained a much higher initial xylan content of 24%, as opposed to 15% in CS and EC. This higher xylan content indicates higher recalcitrance of the feedstock and potentially led to the lower xylose yield in S2. With residual IL inhibiting our fermentation studies in Section 3.2, we relied on literature to estimate fermentation results (Humbird et al., 2011). We assumed 95% and 85% (of theoretical) ethanol yields from glucose and xylose respectively and calculated the individual ethanol yields of each scenario per the original biomass sugar concentrations. Despite significantly lower xylose yield in S2, the calculated ethanol yield was only about 5% (of theoretical) lower compared to those of S1 and S3, i.e. 49.6 Million gallons/year in S2 as opposed to 52 Million gallons/year in S1 and S3. However, any variation in the ethanol yield, even as low as 5%, will directly impact MESP, mandating further optimization of xylan conversion to maintain high yields when incorporating SG into the feedstock blend.

Feedstock prices for the three scenarios were calculated per data provided by LCF and feedstock ratio in the biomass blend composition, provided by predictive model. Per LCF, the estimated prices for EC, SG, and CS on dry basis were $70, 50, and 60/ton, respectively, as reported in Narani et al. (2017). Compared to S1, the effective price of feedstock blend in S2 remained the same but in the case of S3, the price of feedstock blend was 8% higher. Subsequently, compared to S1, the MESP was 3% higher in S3. Interestingly, MESP was even higher, 8% higher, in the case of S2, potentially due to the lower ethanol yield from

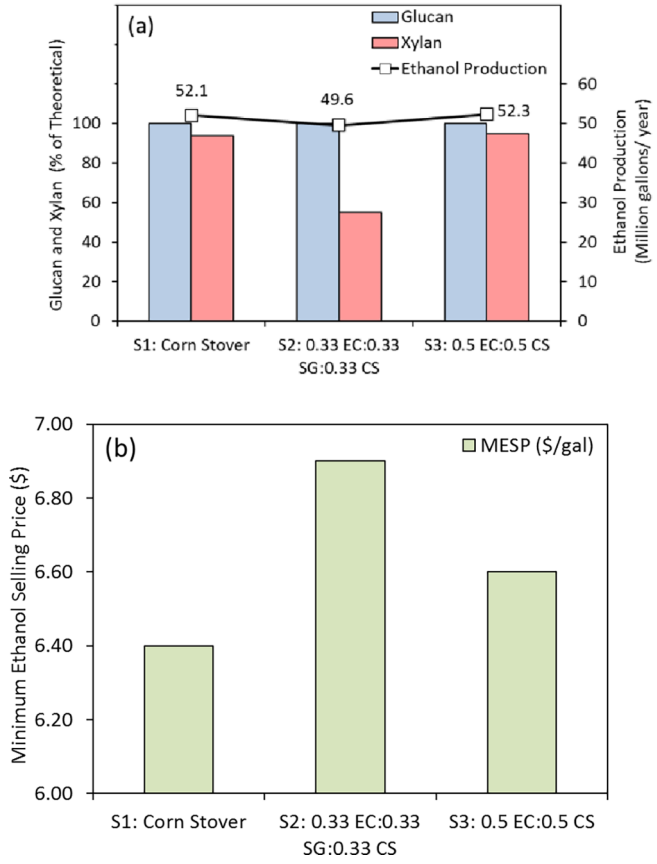


Fig. 5. (a) Primary axis: Glucan and Xylan yields after deconstruction at high-solids loading with IL at different pretreatment conditions; Secondary axis: Ethanol yield estimated by TEA with the assumption of 95 and 85% (of theoretical) ethanol yield from glucose and xylose; (b) Estimated Minimum ethanol selling price (MESP) values for three scenarios with varying feedstock blends.

lower xylan conversion; see Fig. 5(b). Based on this analysis, if pressed to switch from S1 due to supply quantity and/or quality issues, the biorefinery will be more efficient in switching to S3 than to S2, despite higher effective feedstock price in S3. The economic penalty is relatively smaller when switching from S1 to S3 as the increase in MESP is lower at 3% versus 8% penalty associated with a switch from S1 to S2. An ideal scenario for a biofuel producer will be to be able to maintain or improve yields while switching to a biomass blend that is less expensive. However, such an ideal situation may not always be feasible in practice, as evident through this TEA, and therefore choosing an option with less economic penalty is prudent.

Though not central to the main scope of the current work on biomass blends, it is worth noting that the estimated MESP values in the scenarios investigated in this study stayed at around $6.5/gal. This emphasizes the fact that cost-drivers other than the feedstock and product yields exist in an IL-based biorefinery. One such cost driver, worth mulling over in this case, is the energy expended in IL recovery. Specifically, the water needed in the washing step has to be recovered prior to IL recycling. In addition, further improvement in solids loading during enzymatic hydrolysis and minimizing sugar losses during washing could enhance economic performance. As demonstrated recently, the use of biocompatible ILs such as cholinium lysinate ([Ch][Lys]) have the potential to eliminate the need for intermediate washing and separation steps, and thereby improving overall economics (Xu et al., 2016). Biocompatible ILs, like dilute alkali pretreatment in this study, can be carried over into enzymatic saccharification and fermentation, without any impact on yields at scale (Sundstrom et al., 2018). With the afore-mentioned advancements and high ethanol yields (> 75 Gal/dry ton), it is possible to realize cost-competitive biofuels, with an MESP of around $3/gal or less (Konda et al., 2014).

In the long term, Impact analysis and TEA can facilitate the transition of a geographical area towards a large-scale bio-economy with a network of multiple production facilities designed to maximize resource utilization, benefitting both feedstock suppliers and bio-refineries. Blending feedstocks from different sources and suppliers can ensure close to design capacity operation of a biorefinery, throughout its lifetime (of typically 30 years). To this end, in the context of Lee County, the bio-refinery management, farmers, and local government should work towards establishing competitive price structure for local feedstocks (such as, EC and SG in Lee County) to maximize the use of local biomass resources. Although this analysis is specific to the Lee County, due to the nature and blends of feedstocks considered, some of the observations made in this study can be readily applicable to other counties or regions with potential for feedstock blends. The primary cost-drivers and opportunities identified in this study can guide to future advancements towards enabling economically viable cellulosic biorefineries that can process biomass blends throughout the country.

4. Conclusions

Pioneer biorefineries operate under processing conditions optimized for a biomass composition with minimal variability. While this approach is defensible to a limited extent, it can make a biorefinery vulnerable because compositional variability can occur even within a single feedstock, caused by several external factors such as weather patterns, harvesting times and techniques, etc. Through the combination of LCF, predictive model, and TEA in this study, agile tools were developed required to utilize low-cost low-quality feedstock resources across the nation. Lee County was studied as a potential geographical location for a biorefinery.

Acknowledgements

We would like to thank Mr. Kevin Kenney and Dr. Vicki Thompson for providing the guidance required in the initial stages of the project. We would also like to thank Ian Rowe at the Department of Energy for his valuable suggestions during the course of this project. The funding for this work was provided by Department of Energy's Energy Efficiency and Renewable Energy division through BioEnergy Technology Office (BETO). The funding was provided as an annual operating plan to each of the three national laboratories: Lawrence Berkeley, Sandia National Laboratory, and Idaho National Laboratory. Chenlin Li, Allison E. Ray, and Damon S. Hartley would like to thank the United States Department of Energy, Assistant Secretary for the Office of Energy Efficiency and Renewable Energy, Bioenergy Technologies Office for supporting INL under DOE Idaho Operations Office Contract DE-AC07-05ID14157. We thank Travis Schofield and Kevin Hernandez for supporting the nutrient study in this project. We also thank DOE for supporting their Science Undergraduate Laboratory Internships.

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