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Calorimetric Evaluation indicates that Lignin Conversion to Advanced Biofuels is Vital to improving Energy Yields

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Abstract

Energy density measurements using bomb calorimetry were applied along with mass yields to calculate energy yields from combinations of individual processes and lignocellulosic feedstocks. Sample preparation and calorimetric method were fine-tuned for biofuel process pathway prior to measuring the energy density of liquid fuels and catalysts and solid biomass types (untreated, pelletized, pretreated, and enzymatically hydrolyzed). To statistically establish the method, correlation between biomass composition and energy densities were tested. Strong correlations with lignin, hemicellulose, and ash concentrations were observed and statistically validated (Pearson's coefficient, r = 0.92 and -0.81, respectively). Finally, energy densities were applied along with mass yields on a process pathway including ionic liquid pretreatment (6L) and saccharification (2L) of three feedstocks. From switchgrass, eucalyptus, and mixed feedstocks, mass yields of 54.4, 62.0, and 61.7% led to energy yields that were observed to be 59.2, 55.9, and 61.0%, respectively. The disparity in change in mass and energy yields between switchgrass and eucalyptus was identified to have originated from the varied lignin removal during pretreatment. The overall energy recovered from 600 g of switchgrass, eucalyptus, and mixed feedstocks, were 9.8, 10.3, and 10.1 MJ, respectively. Calorimetry can promptly evaluate an integrated multi-process pathway to convert a discrete or mixed feedstock to sugars and other metabolites and eventually to advanced biofuels that can either be a hydrocarbon or a mixture thereof. In this particular study, calorimetry and mass yields indicated that lignin removal led to lower energy yield to liquid fuels.

1. Introduction

The pace of research in advanced biofuels from lignocellulosic biomass has picked up considerably in the past decade. Investigators are moving forward with pilot scale testing of emerging technologies and innovative uses of familiar processes[1, 2]. In the ethanol space, recently, POET-DSM started the operation of Project Liberty, a commercial-scale biorefinery in Emmetsburg, Iowa. The production capacity of Project Liberty is 25 MMGal cellulosic ethanol per year produced through a biochemical process that includes acid pretreatment of corn stover followed by enzymatic hydrolysis and fermentation[3]. Typically, biochemical conversion processes can generate high conversion yields of polysaccharides into ethanol and other advanced liquid fuels, but the energy-dense lignin is left unconverted in the residual solids. These solids are often used as sulfur-free solid fuels, primarily for electricity generation[4]. The lignin-rich residue recovered from Project Liberty's process can be converted through anaerobic digestion to produce up to 2743 MM MJ energy in the form of electricity[3]. Assuming 21.2 MJ in a liter ethanol, the energy released from 25 MM Gal ethanol is equivalent to only 2006 MM MJ, much lower than that being generated from lignin-rich residue from corn stover. Biomass conversion studies have typically focused on mass yields (MY) of precursors and final fuels by presenting mass balances rather than calculating Energy Yields (EY) from biomass[5, 6].

Process-associated energy consumption in biofuels production has been widely studied[7, 8]. While process energy consumption provides an unbiased assessment of process performance, it is not a true representative of energy recovered from the biomass itself and does not represent EY from the process. High EYs and low process energy consumptions are essential for the economic viability of any biorefinery, especially because energy itself is the main product. Even without measuring process energy consumption and by just comparing EYs among various technologies and biomass types, it is possible, in the early stages of research and development, to identify the technologies or the combination of biomass types and technologies that are most likely to yield the greatest economic return at production scale. Also, by comparing EYs from various unit operations, it is possible to minimize unwanted energy losses by altering the course of process development and optimization during earlier stages of scale up. The overall economic performance of a plant can be estimated and enhanced by integrating EY results directly into the early stages of plant design[9].

Energy Density (ED) measurements of individual components of all the streams in the biomass to biofuel process chain can act as the single type of analytical test required to gauge EYs. Moreover,

performing ED measurements on intermediate or final products may serve as an indirect method to ascertain product quality by predicting the compositions through mathematical models that can be established based on calibrations with direct analytical measurements such as chromatography and other gravimetric and wet chemistry assays for components such as sugars and other carbohydrates[10-12]. Such mathematical models will allow researchers and engineers to confidently predict biomass composition but only when the reliability of such predictions are based on precise measurements of ED. Biomass EDs have previously been measured and correlated to elemental and approximate composition, but in these previous studies, oxygen bomb calorimetry was used to measure the ED of loose untreated biomass[13-18]. In this study, to establish the sample preparation of untreated and treated biomass that lead to statistically validated reproducibility, we adapt pelletization, a preparation process derived from methods developed on coal and other solid fuels. To our knowledge, this report is the first to describe the use of this technique and the influence of compression force during pelletization on the precision of ED measurements from biomass samples. Also, our team at the Advanced Biofuels Process Demonstration Unit (ABPDU), was the first to demonstrate the application of such ED measurements to explain EYs of each unit process along with MYs of various biomass components in a scale-up deconstruction study[19].

The ABPDU, in collaboration with the Joint BioEnergy Institute (JBEI), has performed benchmark studies to resolve key issues associated with evaluating EYs in biofuels production. The objective of this study is to establish energy yields from a process pathway using calorimetry. To achieve this goal, we had to fine tune the method to measure calorific values of biomass and liquid samples. Primarily, in this study we pelletized three biomass feedstocks that underwent several treatments[14-16], as opposed to previous attempts at adopting calorimetry that were performed on loose samples. To further ensure that we are able to associate EYs to process changes, we statistically tested the correlations between ED and biomass composition. Once we were able to establish calorimetry as a possible predictive tool of in-process material quality, we applied process mass balance to compute EYs for a process pathway. In the discussion part of the manuscript, we evaluate EY as a metric of interest for bioprocess optimization and establishing comprehensive energy balances. Finally, the concluding perspective provides an insight into the application of precision bomb calorimetry as a useful analytical tool for biofuel process development.

2. Experimental Section

2.1. Biomass Feedstocks, Chemicals, and Enzymes

Five different biomass types, to include agricultural residues, grasses, and woody residues, were tested for ED after various states of biomass deconstruction process. Switchgrass #1, Eucalyptus #1, Corn Stover, Pine, and Eucalyptus were obtained from the Idaho National Laboratory (Idaho Falls, Idaho) and Switchgrass #2 was obtained from University of California - Davis. Along with discrete feedstocks, two mixed feedstock types, eucalyptus and switchgrass in a mass ratio of 1:1 and eucalyptus, switchgrass, corn stover, and pine in a mass ratio of 1:1:1:1 were prepared. The moisture content of all biomass types were less than 10% (w/w) and were accounted prior to preparing mixed feedstocks. The particle size distribution of all biomass types was determined in accordance with ASTM D1511-10, using a sieve shaker (Vibratory Sieve Shaker AS 200, Retsch, Newtown, PA, USA). The majority (54% w/w) of all biomass types yielded particle sizes ranging from 0.1 to 0.6 mm. Further information on biomass types was provided elsewhere[19, 20].

Trifluoroacetic acid (TFA), ethanol, acetic acid, sodium acetate, sulfuric acid, sodium hydroxide, and the monosaccharides used for standards including arabinose, galactose, xylose, glucose, and cellobiose were purchased from Sigma-Aldrich (St. Louis, MO). 1-Ethyl-3-methyl-limidazolium acetate ($[C_2C_1im][OAc]$, BASF, Ludwigshafen, Germany, purity $\geqslant$90%) was used as the IL catalyst in the pretreatment. Novozymes (Davis, CA) generously provided cellulase (CTec2®) and hemicellulase (HTec2®) required for the enzymatic hydrolysis unit process.

2.2. Ionic Liquid Pretreatment and Enzymatic Hydrolysis

While Switchgrass #2 was tested at two solids loading, (i) 10% and (ii) 15% (w/w) biomass in final slurry during IL pretreatment, Switchgrass #1, Corn Stover, Pine, Eucalyptus, and Mixed Feedstocks were treated at 10% (w/w) biomass in final slurry. A Hastelloy C276 10L Parr floor stand reactor (Model# 4556, Parr Instrument Company, Moline, IL) was used to carry out the pretreatments of Switchgrass #1, Eucalyptus, and Mixed Feedstocks at 140°C for 1 hour with constant agitation[19]. A mixture of CTec2® and HTec2® (54 and 6 mg enzyme/g glucan in pretreated biomass, respectively) was used to hydrolyze the 10% (w/w) IL pretreated biomass at 50°C for 72 hours in a 2L constant stirred reactor (IKA LR-2.ST, IKA Works, Wilmington, NC, USA)[21]. The hydrolyzed solids were filtered, washed and recovered via paper filtration and then dried in a vacuum oven (Binder VDL 115, Bohemia, NY, USA) at 45°C overnight. More details on

the preparation, pretreatment, and enzymatic hydrolysis of all biomass types were provided elsewhere[19, 21, 22].

2.3. Biomass Compositional Analysis

Compositional analysis was conducted on all solid streams obtained from the various stages of the biomass conversion chain, including untreated, pretreated, and enzymatically hydrolyzed biomasses. Acid-insoluble lignin and structural carbohydrates were quantified following a two-step sulfuric acid hydrolysis Laboratory Analytical Protocol (LAP) developed at the National Renewable Energy Laboratory (NREL)[23, 24]. Carbohydrates in the liquid fraction of the samples were measured by high performance anion exchange chromatography (Dionex ICS-3000 HPAEC, Sunnyvale, CA, USA). More details on compositional analysis methods were provided elsewhere[19].

2.4. Sample Pelleting and Bomb Calorimetry

The energy content of untreated, pretreated, and enzymatically hydrolyzed biomass were measured using an oxygen bomb calorimeter (IKA C2000, Wilmington, NC, USA), see Figure S1 in supplementary data. Prior to any testing, biomass samples were dried in a vacuum oven at 50°C for 3 hours and Moisture Content (MC%) was determined using a moisture analyzer (Mettler Toledo MJ33, Columbus, OH, USA). After the drying, biomass samples were pressed into pellet form using a hydraulic pelletizer (MTI 12T pelletizer, Richmond, CA, USA). The weights of pelletized samples (W_{sample}) in grams were measured on a precision digital scale (Mettler Toledo, model XP105, Columbus, OH, USA). The pelletizer chamber yielded a constant pellet radius of 0.64 cm; the height of the pellets (H_{sample}) in cm was measured using Vernier calipers (Fowler, model IP65, Brantford, ON, Canada). The weights and volume of the samples was used to calculate the mass density of the pelleted sample, MD_{sample} (kg/m^3).

The bomb calorimeter was calibrated using a known amount of standard benzoic acid (Sigma-Alderich, St. Louis, MO, USA). Intrinsic to the method, ΔT_{std} represents the recorded rise in temperature of benzoic acid during combustion. The term ΔT_{sample} represents the recorded rise in temperature after combustion of the pelleted biomass samples. The ED of solid samples was calculated based on the following equation:

$$ED = ED_{BA} \times \frac{W_{BA} \times \Delta T_{sample}}{W_{sample} \times \Delta T_{std}} \qquad \text{Eq [2]}$$

where, ED is energy density of sample, ED_{BA} is the energy density of benzoic acid, W_{BA} is the weight of benzoic acid, and W_{sample} is the weight of sample.

The use of a standard combustion aid (IKA C10 Acetobutyrate capsules, Wilmington, NC, USA) facilitated the measurement of EDs of liquid samples; see Figure S1 in Supplementary Data. An ignition thread made of 100% cotton was provided by IKA to ignite the solid samples. According to ASTM D5468, correction of heating value of acid combustion is needed when sulfur content in samples contributes significantly to the heat generated during combustion[17, 18, 25, 26]. Therefore, off-site elemental analyses were conducted and sulfur content in all biomass samples was observed to be less than 2.0%, a level negligible for the purposes of this study.

Ash weight was measured by subtracting the weight of the crucible before (W_{cb}, g) and after (W_{ca}, g) bomb calorimetry. After bomb calorimetry, unburned solid residue was heated in a muffle furnace at 1200°C for 12 hours. No weight change was observed, supporting the hypothesis that only ash was left after calorimetry. For the purpose of assessing the organic fraction of the biomass, ED calculations were adjusted by subtracting the moisture and ash content, using the following equation. This adjustment ensured that energy was accounted only to the cellulose, hemicellulose, and lignin fractions of the samples.

$$\mathrm{ED}a = \frac{\mathrm{ED}_{\mathrm{sample}}}{(1-\mathrm{Ash}\%)} \qquad \text{Eq [3]}$$

Three standards were chosen for solid samples: 1) Glucose with particle size less than 75µm (≥99.5 w/w%, part# G8270, Sigma Aldrich, Columbus, OH, USA), 2) Pretreated Eucalyptus with particle sizes between 75 µm and 2mm, and 3) Untreated Eucalyptus with particle sizes larger than 2mm, see Table S1 in Supplementary Data for ED*a* information. Eucalyptus was chosen as the model feedstock to represent biomass. [C_2mim][OAc] and ethanol were chosen for as standards for liquid samples. Ethanol HHV is included here to verify the protocol with a standard value reported elsewhere, which was lower by 1.6% from our measurement[27].

In the case of pre-pelleted biomass samples, where the source material was extrusion pressed prior to shipment, the pellets were ground using mortar and pestle before hydraulically re-pelleting the particulate samples. Even though a change in particle size must have occurred during the grinding in mortar, it would not have influenced ED measurements as only the optimal hydraulic force that varied as a function of particle size. When pressed at the optimal force, ED*a* values do not vary as a function of particle size.

2.5. Statistical Approaches applied to Calculate Sample Sizes and Validate Correlations

The means and standard deviations of calorific values of standard materials, glucose and ethanol, was used to calculate the power (1-β), the probability of avoiding a type II error, of the method according to equation 4. The minimum level of statistical significance for the power calculations, or α, was set at 0.05.

Pearson's product-moment coefficient was calculated to correlate the paired data of a component of biomass (Klason-lignin, non-glucan saccharides, and glucan) and ED of biomass samples, as shown in Equation 5. The sample size required to obtain an acceptable correlation was calculated according to equation 6 based on Fischer's archtan transformation and an acceptable power of 0.80. Correlation coefficient was considered statistically significant only when the calculated p-values were observed to be less than 0.05. All variables were observed to follow normal distribution and the residuals were observed to be independent of the factors tested.

$$1-\beta = Z\left[-Z\left[\frac{\alpha}{2}\right]+\frac{(\mu_{obs}-\mu_{std})\sqrt{n}}{\sigma}\right] \quad \text{Eq. [4]}$$

$$r=\frac{\sum_{i=1}^{n}x_i y_i-\frac{\sum_{i=1}^{n}x_i\sum_{i=1}^{n}y_i}{n}}{\sqrt{\left(\sum_{i=1}^{n}x_i^2-\frac{\left(\sum_{i=1}^{n}x_i\right)^2}{n}\right)\left(\sum_{i=1}^{n}y_i^2-\frac{\left(\sum_{i=1}^{n}y_i\right)^2}{n}\right)}} \quad \text{Eq. [5]}$$

$$n=\left(\frac{Z[\alpha]+Z[\beta]}{\frac{1}{2}\log_e\frac{1+r}{1-r}}\right)^2+3 \quad \text{Eq. [6]}$$

where, 1-β = power of the test or the probability of type II error, α= the probability of type I error, Z(x) = area under the curve to the left of x on a standard normal table, μ_{obs} = mean of calorific value of the samples, μ_{std} = mean of the calorific value of the corresponding standard materials, n = sample size, σ = standard deviation of the calorific value for the corresponding standard materials, x = concentrations of biomass components (w/w%) in each of the biomass solid samples, and y = corresponding ED*a* of the biomass samples.

3. Results and Discussion

3.1. Optimizing Hydraulic Force to obtain Reproducible Measurements from Bomb Calorimetry

To ensure reproducible ED results from biomass samples, a coefficient of variation (CV) of less than 1% for 5 replicate measurements served as the target specification in this study. The pelleting force, that is, the hydraulic force of compression for a given material, was optimized to yield a highly reproducible Higher Heating Value (HHV) from solid samples. Particle size appeared to have greatly influenced the optimal hydraulic force, see Table S1 in Supplementary Data. If the pelleting force was too low, the pellet would often splash during combustion and a substantial fraction of material would remain unburned in the crucible after calorimetric measurement, see Figure S1 in supplementary data. If the pelleting force was too high, the pellet would not combust completely, also resulting in a failed measurement. Excessive pressure could have resulted in material densities that prevented oxygen from diffusing into the pellet during combustion. The optimal mass densities for all the three solids varied substantially indicating that there is no universally applicable optimal mass density for all sample types, which yields reproducible calorimetry. With the appropriate pelletizing force, the CVs of 5 repeated calorimetric measurements for all the solids were observed to be well within the 1% CV target for the study. Sample preparation and CV for liquid samples were also established, as ED measurements of solid samples could include contribution from solvents that seep into the solids during experimental studies. These methods ensured reproducible data from process samples and allowed us to attribute variations in ED data to the varying compositions of the biomasses.

3.2. Paradoxical Influence of Ash on Energy Density of Biomass

EDs were recorded for 33 samples of biomass from various processes in the biomass deconstruction sequence. Table 1 lists all the samples tested in this study, along with relevant information: process conditions, ash contents, and measured EDs. The list includes 9 single-source biomass samples and 10 biomass types, in either loose or pre-pelleted forms, with varying ash contents. The statistical power, (1-β), of the calorific measurement tests for all samples was calculated based on glucose and eucalyptus as standard materials. Power was observed to be 100% for all the 10 biomass types and 33 samples assuring that the method provides an accurate measure of ED for the various biomass samples, and one sample is enough to obtain a representative value.

Generally, EDs of untreated biomass types followed the order: pine > eucalyptus > corn stover > switchgrass. Woody feedstocks averaged an ash content of 3.8% (w/w), whereas the herbaceous

feedstocks averaged 6.7% (w/w). Consistent with earlier observations, ash content had a diluting influence on biomass ED[14]. IL pretreatment did not substantially reduce the ash concentration in residual switchgrass when pretreatment (PT1 and PT2) was conducted at high solid loadings (15% w/w). However, at a lower solid loading of 10% (w/w), pretreatment (PT3) led to a large drop in ash content from 5.2% (w/w) to 0.5% (w/w) in eucalyptus and from 6.3% (w/w) to 2.7% (w/w) in switchgrass. The ED of both eucalyptus and switchgrass increased, which appeared to be largely due to loss of ash. It is possible that lower solid loading enhanced gel homogenization after IL pretreatment, resulting in a partial loss of ash during the subsequent wash steps. When ED was adjusted by excluding the weight of ash from the total weight of the biomass sample, ED*a* of the "ash-free" untreated and pretreated biomass samples do not vary substantially. This indicates that the IL pretreatment itself does not influence ED*a* of residual solids, if tested on an ash-free feedstock. However, it is unrealistic to expect an ash-free feedstock for a biorefinery. Moreover, pretreatment is devised not only to break the lignin cell wall but also to remove various inhibitors, ash among them, to improve performance of downstream enzymatic and fermentation treatments.

Even though the overall mass of ash was reduced, concentration of ash in the solid residue recovered after pretreatment and enzymatic hydrolysis increased. Surprising, solid residues after enzymatic hydrolysis were observed to have the highest ED*a*s, even after correcting for the substantial ash concentration in the samples. This observation suggested that ED*a* measurements are more profoundly influenced by factors, other than ash content. Compositional analysis of biomass was conducted to better understand the influence of these factors.

3.3. Correlation of Biomass Energy Density with Lignin and Saccharides

In this study, saccharides were quantified and categorized into glucan and non-glucans. Glucan was used as a representative of cellulose, and non-glucan was used as an estimation of hemicellulose, which included xylan, arabinan, and galactan. Klason lignin was used as a representative of lignin, both acid soluble and insoluble, in the biomass. A strong positive linear correlation ($R^2 = 0.85$) was found between the ED*a* and the Klason lignin concentration in residual solids (Figure 1a). A Pearson's coefficient (r) of 0.92 with statistical significance (p-value < 0.0001) further buttresses the strong correlation. The sample size calculation, assuming a statistical power of 0.80, suggests that only 3 samples were required to establish the correlation, well within the sample size used in this study, 33. A weighted correlation factor was calculated and results show that all but one (enzymatically hydrolyzed biomass sample) did not follow the strong correlation between lignin and energy density.

A negative but a strong linear correlation, with an r value of - 0.81 and p-value < 0.01, was found between non-glucan saccharides concentration and ED*a*, as shown in Figure 1b. The required sample size assuming a power of 0.80 was, again, calculated to be much lower than required at 4. The computed weighted correlation factor also indicated the strong correlation with 27 of the 33 samples following the trend. Glucan concentration did not correlate very well with ED*a*, Figure 1c. Even though the p-value (< 0.01) and sample size calculation ($n_{required} \geq 9$) indicated a significant possibility of a correlation, both the r value and R^2 were low at - 0.44 and 0.53, respectively when data was fitted to a linear model. Also, more than a fourth of the samples (9 of 33) did not follow the trend of the weighted correlation factor. The weighted correlation factors were reviewed for all treatment effects. However, none of the treatments seemed to have a consistent effect on any of the correlation.

Since the sample sizes required to establish the correlations were found to be much lower level than 33, we subdivided the data based on treatments to better understand the influence of biomass compositions on ED*a*. The samples, listed in Table 1, were divided into four subsets (untreated, pelletized, pretreated, and enzymatically hydrolyzed with 5, 4, 15, and 9 samples, respectively) and linear correlations between biomass compositions and ED*a* were calculated separately for biomass from each treatment type, listed in Table 2. Expectedly, the results indicated that Klason lignin concentrations in biomass correlated well with ED*a*, regardless of the treatment type. However, only the correlation between pretreated biomass and ED*a* exhibited a statistically significant r (= 0.91) and p-value (< 0.00001). Also, the calculated sample size required to establish this correlation ($n_{required} \geq 6$) was much lower than the applied ($n_{tested} = 15$). The same was not true for any of the Klason lignin correlations from other treatment types ($n_{required} \geq 7$, 10, and 12 and $n_{tested} = 5$, 4, and 9 for untreated, pelletized, and enzymatically hydrolyzed biomass types, respectively), potentially leading to correlations unsuitable for calibrations. The Klason-lignin concentrations of pretreated solids were more distributed along the range of Klason-lignin measurements, see Figure 1a, possibly causing the strong correlation. While Klason-lignin correlated well for only one treatment type, none of the other biomass components correlated with any treatment types.

Non-glucan saccharide concentrations and ED*a* followed a negative linear relationship with very high r values and in two cases, pelletized (-0.98) and pretreated (-0.88) biomass types, the sample sizes applied were equal or higher than the required sizes. However, the high p-values (p>0.01) render these correlations inapplicable for analytical calibrations. In the case of correlations between glucan concentration and ED*a*, as expected, linear correlations were weak for samples from all treatment types,

with R^2 value as low as $6E^{-05}$ for pelletized biomass. There was a negative correlation between glucan concentration and ED*a* in IL pretreated biomass but a positive correlation between glucan concentration and ED*a* in other sources. The calculated sample size to obtain a reliable correlation was much higher than the sample sizes applied for all treatment types. Overall, correlation assessment between glucan concentration and ED*a* was inconclusive. The hydrogen bonding in glucan is substantially varied before and after IL pretreatment and hydrogen bond concentration can contribute extensively to the ED*a* of a sample. Surprisingly, the glucan concentration of untreated samples, with maximum hydrogen bonding, showed least correlation with ED*a* values, whereas, glucan concentration of pretreated samples correlated better, even statistically significant at p-value = 0.015, with ED*a*. Untreated but pelletized and enzymatically hydrolyzed samples ranged between these two treatment types. It can be supposed that lower lignin content in samples, as was the case with samples after IL pretreatment, is required to avoid interference in glucan's correlation with ED*a*.

While this theory needs further investigation, the ED*a* measurements were nonetheless quite accurate for each of the samples. Even though only one ED*a* correlation, with K-lignin concentration in IL pretreated biomass, can provide a reliable calibration, the ED*a* measurements of each of the samples could be used along with MYs to calculate EYs for the corresponding processes.

3.4. Mass Balance and Energy Yield in Scale-up Case Studies of IL Pretreatment and Enzymatic Hydrolysis

Three runs of IL pretreatment (at 6L scale) using $[C_2C_1im][OAc]$ and enzymatic hydrolysis (at 2L scale) were conducted on three feedstock types: switchgrass #1, eucalyptus, and mixed biomass [19]. Figure 2 is a depiction of mass balance of and EY from this process on switchgrass #1 and eucalyptus. Biomass was introduced into the process chain along with IL (Stream 1) and heated to 140°C for 3 hours to break the cell walls of biomass. Liquid (Stream 2) and solid (Stream 3) fractions from pretreatment were separated after homogenization and centrifugation steps. Energy in biomass was split into these two streams. The liquid stream contained major fraction of lignin dissolved in the $[C_2C_1im][OAc]$, along with small amounts of hemicelluloses and cellulose. The rest of biomass in the solid fraction, primarily rich in cellulose, was then used as the feedstock in enzymatic hydrolysis. After enzymatic hydrolysis, mass and energy in recovered solid fraction after pretreatment (Stream 3) were further split into liquid and solid fractions. Most saccharides were converted into monosaccharides located in the liquid stream (Stream 6), while part of unreacted lignin and insoluble solid remained in the solid stream (Stream 5). The product stream (Stream 6) represents the mass and energy from biomass that was eventually

converted through a bio-chemical process to ferment biomass sugars and produce biofuel (Lucas et al., 2014).

Energy in each of the solid stream numbers 1, 3, and 5 was calculated as a product of the mass of the biomass released into the steam along with its ED*a*. Energy in liquid streams 2 and 6 were calculated as the difference between the energy input and energy output in the solid streams associated with the unit process. The product stream (Stream 6) represents the mass and energy from biomass that was eventually experimentally evaluated through a bio-chemical process, where the biomass sugars were fermented to biofuel[28]. While streams 2 and 5 also are product streams, they were not experimentally reclaimed in this or other studies. However, hemicellulosic sugars in stream 2 can be converted to advanced biofuels, and recent research indicates that low-molecular weight lignin can also be converted through bio-chemical processes to advanced biofuels[29, 30]. Furthermore, stream 5 contained lignin in the residual solid that can directly, without any further processing, replace coal for electricity production[4, 29, 30].

MY from switchgrass (68.0% theoretical) after IL pretreatment was lower than EY (61.6% theoretical). In contrast, EY (68.0% theoretical) after IL pretreatment of eucalyptus, was lower than MY (74.3% theoretical), see Figure 2 (b). This disparity was primarily due to the higher ED*a* of untreated eucalyptus, possibly due to the higher Klason-lignin concentration in eucalyptus at 32.5% compared with that in untreated switchgrass at 22.1% (w/w). In spite of higher lignin removal during IL pretreatment of eucalyptus, stream 3 carried more energy into the enzymatic hydrolysis process. Again, due to the lignin removal, stream 2 for switchgrass carried lower EY out of pretreatment process but at a much higher rate than MY. In the case of mixed feedstock, there is very little variation between the two parameters after IL pretreatment; EY and MY = 70.3 and 70.4%, respectively, see Figure 2 (c). MY and EY for both feedstocks through enzymatic hydrolysis was similar, probably due to the strong influence of pretreatment rendering feedstocks similar to this unit operation. While EY in the form of sugars in this process was higher for switchgrass than for eucalyptus, the total energy recovered after IL pretreatment and enzymatic hydrolysis of 600 g of eucalyptus was 7.9 MJ compared to 7.3 MJ from 600 g switchgrass[21]. This anomaly is, again, primarily due to the higher initial lignin concentration and thereby higher ED*a* of eucalyptus than that of switchgrass. The ED of bisabolane, a C15 alkane and an advanced biofuel, can be assumed to be that of biodiesel at 48 MJ/kg, and the ED of ethanol has been measured in this study to be 29.2 MJ/kg[31]. The theoretical conversion rates of glucose to bisabolane and ethanol are 25.4 and 51.0%, respectively[32]. If all the sugars in stream 6 of switchgrass were converted at a

theoretical rate to ethanol, we would obtain MY and EY of 18.0 and 29.5% (theoretical). But when converting the sugars to bisabolane at a theoretical rate, the MY would be much lower at 9.0%, even though the EY value would be comparable to that from ethanol at 24.2%. Production of higher quality or energy-dense liquid fuels from renewable sources at EYs, not MYs, comparable to that from lower quality fuels is vital for the current transportation infrastructure, especially in the aviation industry [31]. By incorporating ED*a* measurements to sugar MYs, we were able to understand EYs from the entire deconstruction process and extrapolate the possible outcomes from fermentation systems. In the future, through this founding study, calorimetry can be used to identify the ideal feedstock or mixture of feedstocks to obtain maximum EY and thereby maximum economical return from a single or several biofuel production pathways.

3.5. Perspective

Fermentable sugars can be converted to ethanol or an advanced biofuel that can be readily incorporated into the current infrastructure. Precision bomb calorimetry is useful in measuring a single analytical characteristic, ED, of such fuels, because they are often comprised of mixtures of hydrocarbons, rather than pure, single molecules. Unrefined biofuels, those which have not been distilled or otherwise purified to meet final specifications, may carry several components derived from the process chain, especially elements such as chloride, sulfur, and nitride. While such components may be not be concentrated in the liquid output streams, they may have a significant impact on the ED of the biofuel and thereby should be assessed when trying to establish mass and energy balances for the entire conversion system. Bomb calorimetry provides a rapid and accurate assessment of a single advanced biofuel or mixture of fuels.

Investigators are only beginning to consider integrated processes for production and recovery of advanced biofuels. Individual unit operations, studied in isolation, that are highly effective (> 90% conversion) may not lead to a high mass fuel upon integration for a complete production chain; 6 processes at 90% conversion rate will yield 54% overall conversion. This, in addition to low-value electricity generation from lignin, can potentially lead to lower MYs and EYs than desired for reasonable economic returns from a biorefinery. It is necessary to maximize the conversion of all energy stored in biomass to high-quality fuels and co-products, primarily by the high-efficiency conversion of fractionated lignin to energy-dense liquid fuels or other chemicals. Chemical pathways for such conversions already exist and are more well-studied than bio-chemical pathways that are being invented to ferment low molecular weight lignin [30, 33-35]. Lignin is not a single molecule and chromatographic

measurements of the several molecules of lignin and their conversion to several hydrocarbons for MY measurements can be complicated and tedious. Precision bomb calorimetry can be a very useful tool in such cases, where a single analytical technique can be applied to advanced biofuel production pathways that produce multiple hydrocarbons from several components, such as polysaccharides and fractionated lignin molecules. Energy yield, along with mass yield of precursors and fuel, is an informative parameter that is required to assess novel biofuel production pathways. Bomb calorimetry is a simple, accurate, and precise analytical technique that provides the measured, not calculated, EY values for traditional and advanced pathways alike.

4. Conclusion

We developed a method to measure energy densities (ED) of several process samples obtained from a biofuel production process chain. The method exhibited less than 1% coefficient of variation over repeated measurements for various standards giving a 100% statistical power for samples from several feedstocks, indicating that ED measurements adjusted for ash, ED*a*, was accurate for each sample. The strong correlation between lignin concentrations in pretreated solids and ED*a* was observed to be valid mathematical correlations. ED*a* of the solid output stream after pretreatment decreased but increased after enzymatic saccharificiation, primarily due to the influences of ash and lignin concentrations, respectively. Finally, we were able to use this analytical method to establish EY as a function of mass yield (MY) of fermentable sugars from biomass conversion.

5. Acknowledgements

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List of Tables

403 Table 1. Energy density and Ash content of biomass samples

Sample #	Biomass Type	Treatment*	Ash (% w/w)	Energy Density, ED (MJ/kg)	"Ash-free" Energy Density, ED*a* (MJ/kg)
1	Eucalyptus	UN	5.22 ± 0.10	19.48	19.61
2	Switchgrass #1	UN	6.26 ± 0.20	17.39	18.22
3	Switchgrass #2	UN	9.63 ± 0.20	17.82	18.38
4	Lodgepole Pine	UN	6.70 ± 1.20	18.79	19.45
5	Eucalyptus pellet	UNP	1.21 ± 0.32	19.25	19.02
6	Switchgrass pellet	UNP	4.47 ± 0.24	18.31	17.49
7	Corn Stover pellet	UNP	6.26 ± 0.22	17.96	16.84
8	Lodgepole Pine pellet	UNP	1.95 ± 0.27	19.59	19.21
9	Biomass Mix 1‡	UN	3.82 ± 0.20	18.57	17.86
10	Switchgrass #2	PT 1	5.42 ± 0.10	15.88	16.79
11	Switchgrass #2	PT 1	5.20 ± 0.09	16.63	17.54
12	Switchgrass #2	PT 1	5.89 ± 0.23	17.35	18.43
13	Switchgrass #2	PT 2	4.44 ± 0.08	16.56	17.33
14	Switchgrass #2	PT 2	4.93 ± 0.59	16.99	17.87
15	Switchgrass #2	PT 2	6.15 ± 0.04	17.63	18.79
16	Eucalyptus	PT 3	0.49 ± 0.15	19.73	19.83
17	Eucalyptus	PT 3	0.38 ± 0.08	19.71	19.79
18	Eucalyptus	PT 3	0.44 ± 0.08	19.61	19.69
19	Switchgrass #1	PT 3	2.74 ± 0.13	17.79	18.29
20	Switchgrass #1	PT 3	2.48 ± 0.52	17.78	18.23
21	Switchgrass #1	PT 3	1.86 ± 0.20	17.86	18.20
22	Biomass Mix 2‡	PT 3	1.31 ± 0.23	18.94	19.20
23	Biomass Mix 2‡	PT 3	1.05 ± 0.19	18.69	18.89
24	Biomass Mix 2‡	PT 3	1.22 ± 0.22	18.40	18.63
25	Eucalyptus	EH	0.57 ± 0.05	22.15	22.28
26	Eucalyptus	EH	0.60 ± 0.01	22.34	22.48
27	Eucalyptus	EH	0.60 ± 0.03	21.67	21.80
28	Switchgrass #1	EH	8.78 ± 0.03	18.43	20.20
29	Switchgrass #1	EH	8.83 ± 0.03	18.62	20.43
30	Switchgrass #1	EH	8.70 ± 0.02	19.95	21.85
31	Biomass Mix 2‡	EH	2.65 ± 0.12	21.44	22.03
32	Biomass Mix 2‡	EH	2.49 ± 0.09	19.79	20.30
33	Biomass Mix 2‡	EH	2.55 ± 0.05	19.82	20.34

404 Notes:

405 ‡Biomass Mix 1: Mass ratio of Eucalyptus: Switchgrass: Corn Stover: Pine= 1:1:1:1; Biomass Mix 2: Mass ratio of Eucalyptus:
406 Switchgras = 1:1

407 *UN is Untreated, UNP is Untreated but Pelletized, PT is Pretreated, and EH is Enzymatic Hydrolyzed biomass samples. The
408 treatment reaction conditions (Reaction Temperature, Reaction Time, Solids Concentration, and Catalyst Loading) for PT 1 are
409 160°C, 3hrs, 15% (w/w); PT 2 are 120°C, 3hrs, 15% (w/w); PT 3 are 140°C, 3hrs, 10% (w/w); and EH are 50°C, 72 hrs, and 10%
410 (w/w) with an enzyme loading of 54mg and 6mg of CTec2 and HTec2 /g glucan in pretreated solid.

411

412 Table 2. Correlation between Concentrations of Biomass Components (w/w %) and
413 Energy Density (ED*a*) (MJ/kg)

Specific component of biomass	Untreated (UN)	Pelletized (UNP)	Pretreated (PT)	Enzymatically hydrolyzed (EH)
	n_{tested} = 5	n_{tested} = 4	n_{tested} = 15	n_{tested} = 9
Klason Lignin	y = 0.13x + 15.19	y = 0.09x + 16.16	y = 0.10x + 16.70	y = 0.21x + 11.41
	R^2 = 0.60	R^2 = 0.77	R^2 = 0.83	R^2 = 0.55
	r = -0.88	r = 0.78	r = 0.91	r = 0.74
	$n_{required} \geq 7$	$n_{required} \geq 10$	**$n_{required} \geq 6$**	$n_{required} \geq 12$
	p-value > 0.01	p-value > 0.01	**p-value < 0.01**	p-value > 0.01
	= 0.05	= 0.22	**< 0.00001**	= 0.02
Non-glucan sugars	y = -0.12x + 22.10	y = -0.12x + 21.78	y = -0.14x + 21.50	y = -0.30x + 25.07
	R^2 = 0.96	R^2 = 0.72	R^2 = 0.78	R^2 = 0.55
	r = -0.85	r = -0.98	r = -0.88	r = -0.74
	$n_{required} \geq 8$	$n_{required}$ **≥ 4**	$n_{required}$ **≥ 7**	$n_{required} \geq 12$
	p-value > 0.01	p-value > 0.01	p-value > 0.01	p-value > 0.01
	= 0.07	= 0.02	= $1.2E^{-05}$	= 0.02
Glucan	y = 0.11x + 14.30	y = 0.01x + 18.57	y = -0.13x + 25.66	y = 0.07x + 19.07
	R^2 = 0.26	R^2 = $6E^{-05}$	R^2 = 0.38	R^2 = 0.04
	r = 0.01	r = 0.51	r = -0.62	r = 0.19
	$n_{required} \geq 125761$	$n_{required} \geq 28$	$n_{required} \geq 18$	$n_{required} \geq 217$
	p-value > 0.01	p-value > 0.01	p-value > 0.01	p-value > 0.01
	= 0.99	= 0.49	= 0.02	= 0.63

414

415 Note: ED*a* is Energy Density of a sample after adjusting for ash and moisture contents
416 n is sample size, y is ED*a*, x is the concentration (w/w %) of the corresponding biomass, R^2 is the coefficient of determination, r
417 is Pearson's coefficient, and p-value represents the statistical significance of correlation. Sample size was calculated assuming a
418 power of 0.80.

419

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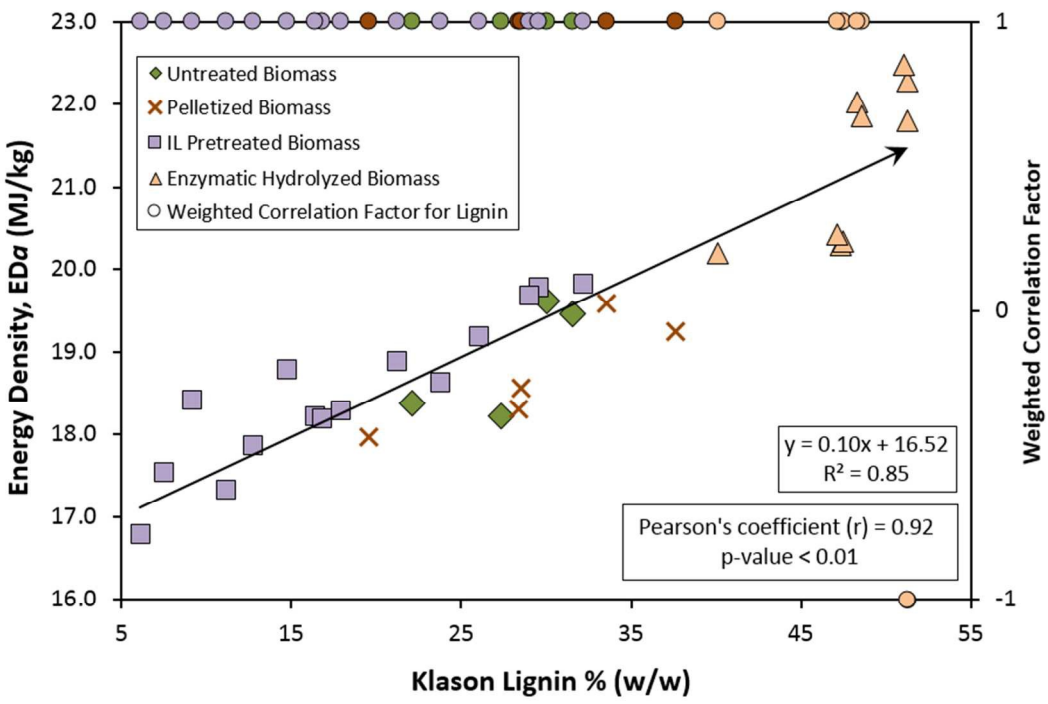
426



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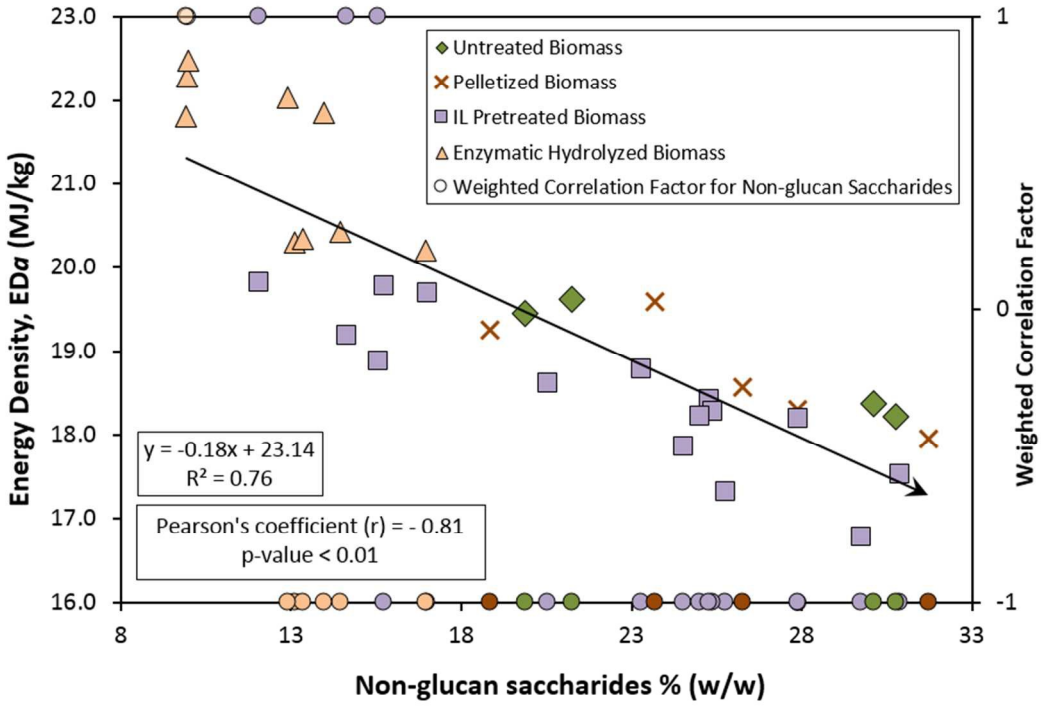
428 (a)



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430 (b)

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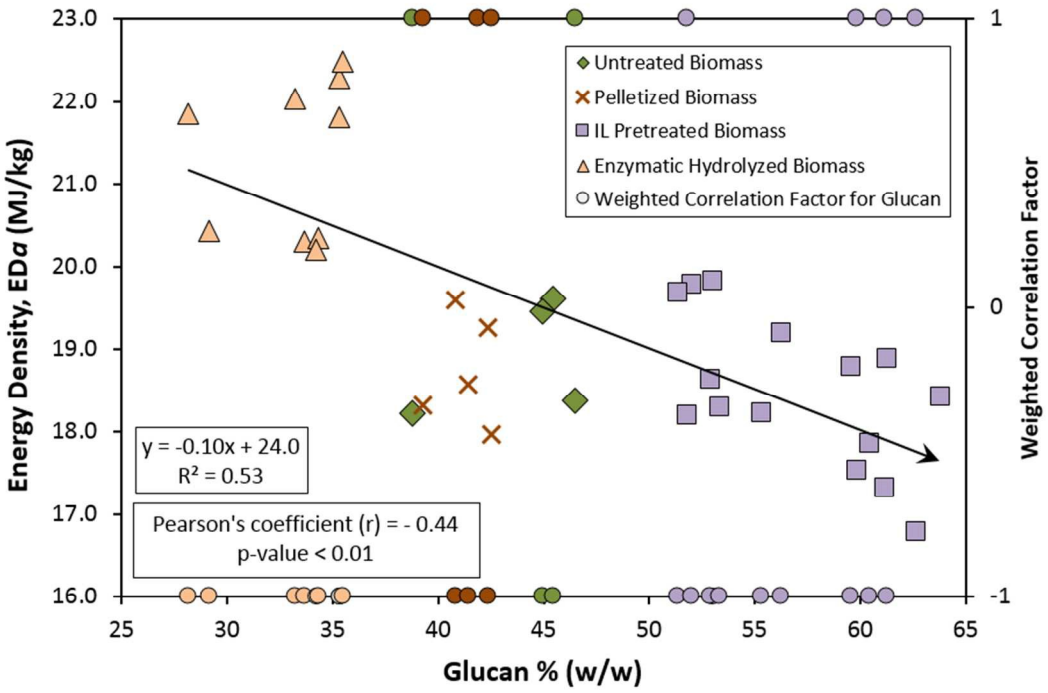
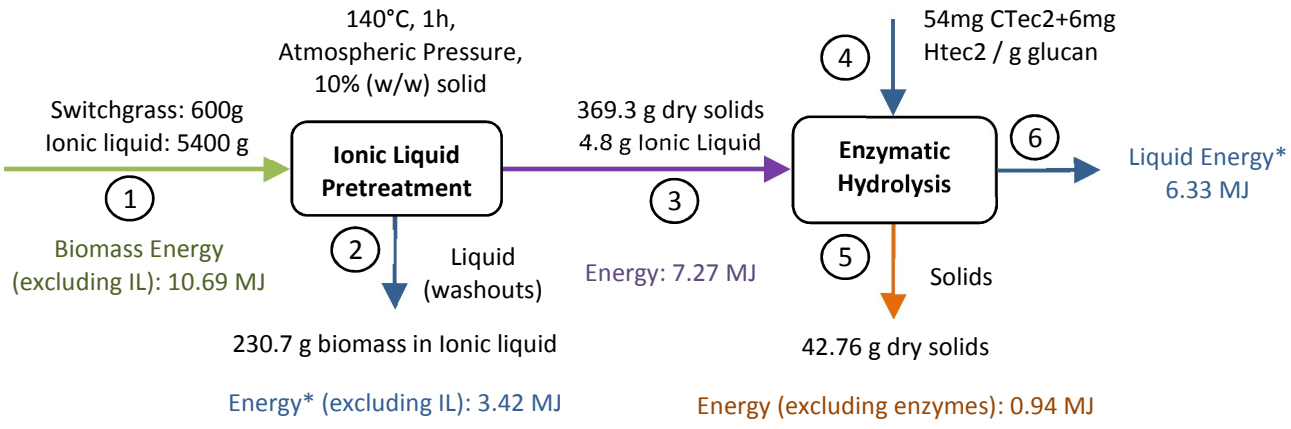


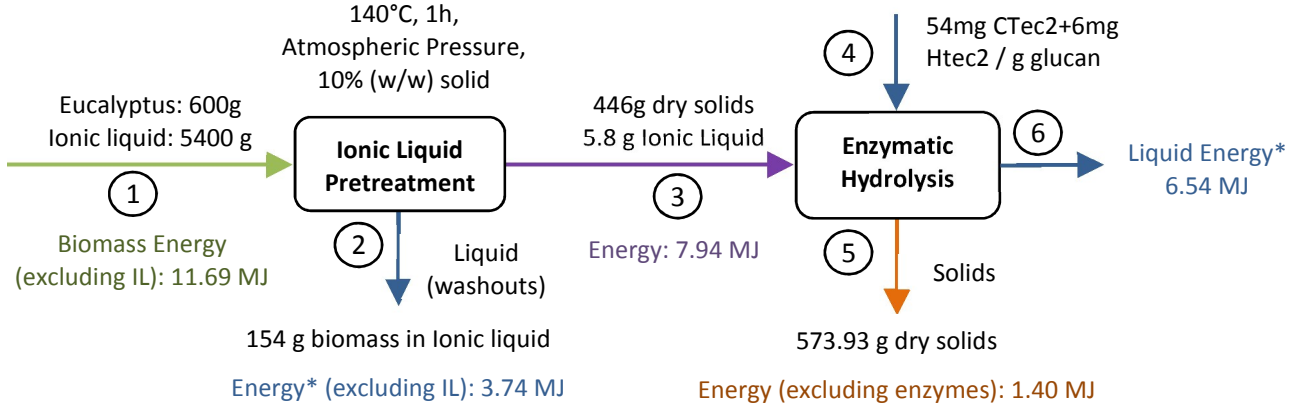
(c)

Figure 1. Correlation of energy density to (a) Klason lignin, (b) non-glucan, and (c) glucan concentration in biomass samples from various treatments; ED*α* is Energy Density of a sample after adjusting for ash and moisture contents



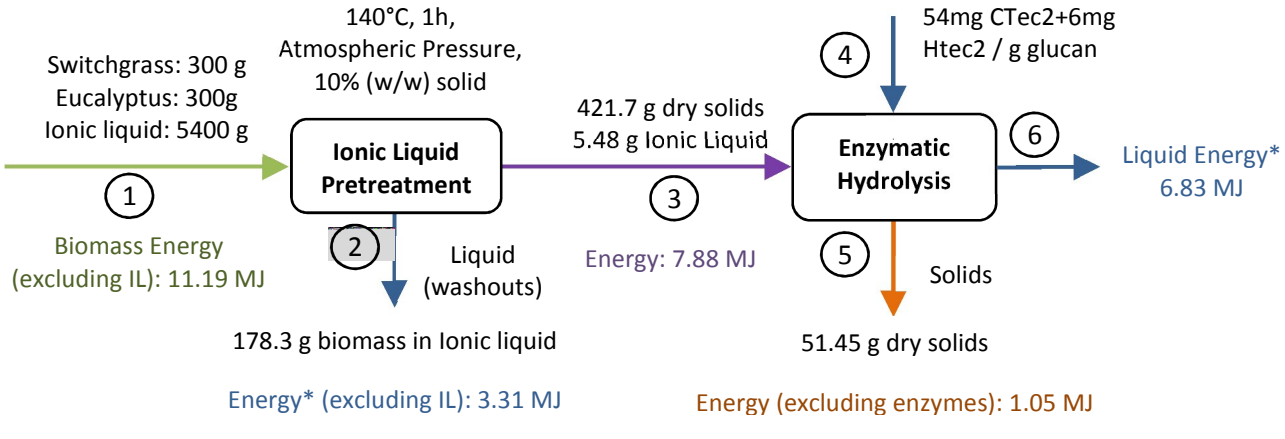
(a)

Mass recovery after IL pretreatment: 61.55%
Energy recovery after IL pretreatment: 67.99%
Mass recovery after Enzymatic Hydrolysis: 88.42%
Energy recovery after Enzymatic Hydrolysis: 87.03%
Overall mass recovery: 54.43%
Overall energy recovery: 59.17%



(b)

Mass recovery after IL pretreatment: 74.33%
Energy recovery after IL pretreatment: 67.96%
Mass recovery after Enzymatic Hydrolysis: 83.42%
Energy recovery after Enzymatic Hydrolysis: 82.31%
Overall mass recovery: 62.01%
Overall energy recovery: 55.94%



Mass recovery after IL pretreatment: 70.28%
Energy recovery after IL pretreatment: 70.38%
Mass recovery after Enzymatic Hydrolysis: 87.80%
Energy recovery after Enzymatic Hydrolysis: 86.71%
Overall mass recovery: 61.71%
Overall energy recovery: 61.03%

(c)

Figure 2. Mass balance and energy yields from (a) switchgrass #1, (b) eucalyptus, and (c) mixed feedstocks after [C_2mim][OAc] pretreatment and subsequent enzymatic hydrolysis; *calculated values

Note: All energy values are reported after adjusting energy density for ash and moisture content in the sample

References

1. B. A. Simmons, *Biofuels*, 2011, **2**, 5-7.
2. T. R. Brown and R. C. Brown, *Biofuels, Bioproducts and Biorefining*, 2013, **7**, 235-245.
3. Energy.Gov, Project LIBERTY biorefinery starts cellulosic ethanol production.
4. C. D. Scown, A. A. Ghokale, P. A. Willems, A. Horvath and M. T. E., *Environmental Science and Technology*, 2014, **48**.
5. R. J. Garlock, V. Balan, B. E. Dale, V. R. Pallapolu, Y. Y. Lee, Y. Kim, N. S. Mosier, M. R. Ladisch, M. T. Holtzapple, M. Falls, R. Sierra-Ramirez, J. Shi, M. A. Ebrik, T. Redmond, B. Yang, C. E. Wyman, B. S. Donohoe, T. B. Vinzant, R. T. Elander, B. Hames, S. Thomas and R. E. Warner, *Bioresource Technology*, 2011, **102**, 11063-11071.
6. C. Wyman, B. Dale, R. Elander, M. Holtzapple, M. Ladisch and Y. Y. Lee, *Bioresource Technology*, 2005, **96**, 2026-2032.
7. J. Y. Zhu and X. J. Pan, *Bioresource Technology*, 2010, **101**, 4992-5002.
8. J. Y. Zhu, W. Zhu, P. Obryan, B. Dien, S. Tian, R. Gleisner and X. J. Pan, *Applied Microbiology and Biotechnology*, 2010, **86**, 1355-1365.
9. J. Hill, E. Nelson, D. Tilman, S. Polasky and D. Tiffany, *Proceedings of the National Academy of Sciences*, 2006, **103**, 11206-11210.
10. L. Murphy, K. Borch, K. C. McFarland, C. Bohlin and P. Westh, *Enzyme and Microbial Technology*, 2010, **46**, 141-146.
11. A. Demirbas, *Energy Conversion and Management*, 2001, **42**, 183-188.
12. A. Demirbas, *Energy, Exploration & Exploitation*, 2002, **20**, 105-111.
13. L. Núñez-Regueira, Rodrı, amp, x, J. A. guez-Añon, Proupı, amp, x, J. n-Castiñeiras, A. Vilanova-Diz and N. Montero-Santoveña, *Thermochimica Acta*, 2001, **371**, 23-31.
14. J. Parikh, S. A. Channiwala and G. K. Ghosal, *Fuel*, 2005, **84**, 487-494.
15. C. Sheng and J. L. T. Azevedo, *Biomass and Bioenergy*, 2005, **28**, 499-507.
16. Y. Uemura, W. Omar, T. Tsutsui, D. Subbarao and S. Yusup, *Journal of Applied Sciences*, 2010, **10**, 3250-3256.
17. A. International, in *D5468-02* 2007.
18. A. International, in *D240*, 2009.
19. C. Li, Tanjore D., W. He, J. Wong, J. L. Gardner, K. L. Sale, B. A. Simmons and S. Singh, *Biotechnology for Biofuels*, 2013, **6**, 1-13.
20. C. Li, D. Tanjore, W. He, J. Wong, J. L. Gardner, V. S. Thompson, N. A. Yancey, K. Sale, B. A. Simmons and S. Singh, *BioEnergy Research*, 2014, **Submitted to**.
21. D. Tanjore, C. Li, W. He, J. Wong, J. Gardner, K. Sale, S. Singh and B. Simmons, presented in part at the 35th Symposium on Biotechnology for Fuels and Chemicals, Portland, OR, USA, 2013.
22. J. Shi, Thompson V. S., Yancey N. A., Stavila V., Simmons B. A. and S. Singh, *Biofuels*, 2013, **4**, 63-72.
23. A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter and D. Templeton, *NREL Analytical Procedure*, 2004.
24. A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter and D. Templeton, *NREL Analytical Procedure*, 2004.
25. N. Bech, P. A. Jensen and K. Dam-Johansen, *Biomass and Bioenergy*, 2009, **33**, 534-537.
26. A. Friedl, E. Padouvas, H. Rotter and K. Varmuza, *Analytica Chimica Acta*, 2005, **544**, 191-198.
27. R. H. W. Maas, R. R. Bakker, A. R. Boersma, I. Bisschops, J. R. Pels, E. de Jong, R. A. Weusthuis and H. Reith, *Biotechnology for Biofuels*, 2008, **1**, 1-13.
28. L. S. Parreiras, R. J. Breuer, R. A. Narasimhan, A. J. Higbee, A. L. Reau, M. Tremaine, L. Qin, L. B. Willis, B. D. Bice, B. L. Bonfert, R. C. Pinhanco, A. J. Balloon, N. Uppugundla, T. Liu, C. Li, D.

Tanjore, I. M. Ong, H. Li, E. L. Pohlmann, J. Serate, S. T. Withers, B. A. Simmons, D. A. Hodge, M. S. Westphall, J. J. Coon, B. E. Dale, V. Balan, D. H. Keating, Y. Zhang, R. Landick, A. P. Gasch and T. K. Sato, *PLoS ONE*, 2014, **9**, 1-17.

29. J. He, Y. Wu and F. Xin, *Applied Microbiology and Biotechnology*, 2014, **doi:10.1128/AEM.00337-14**.
30. J. G. Linger, D. R. Vardon, M. T. Guarnieri, E. M. Karp, G. B. Hunsinger, M. A. Frandern, C. W. Johnson, G. Chupka, T. J. Strathmann, P. T. Pienkos and G. T. Beckham, *Proceedings of the National Academy of Sciences*, 2014, **111**.
31. N. Savage, *Nature*, 2011, S9-S11.
32. P. P. Peralta-Yahya, F. Zhang, S. B. del Cardayre and J. D. Keasling, *Nature*, 2012, **488**, 320-328.
33. C. Xu, R. A. D. Arancon, J. Labidi and R. Luque, *Chemical Society Reviews*, 2014, **43**.
34. A. J. Ragauskas, G. T. Beckham, M. J. Biddy, R. Chandra, F. Chen, M. F. Davis, B. H. Davison, R. A. Dixon, P. Gilna, M. Keller, P. Langan, A. K. Naskar, J. N. Saddler, T. J. Tschaplinski, G. A. Tuskan and C. E. Wyman, *Science*, 2014, **344**.
35. M. Kosa and A. J. Ragauskas, *Green Chemistry*, 2013, **15**, 2070-2074.