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Chemical Characterization and Conversion Assessment of BurCell® System Treated MSW Materials

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Introduction

Cornerstone Resources has developed a proprietary advanced preprocessing technology, the *BurCell*® System, for production of a high quality organic biomass feedstock from numerous waste resources. While suitable for a variety of feedstock, the focus of development for the *BurCell*® System has been thus far municipal solid waste (MSW). In MSW, the system technology is able to capture non-recyclable waste paper, cardboard, and organic materials, homogenize these materials and allow for separation of the non-organic fraction. The resulting homogenized organic material typically represents in excess of 50% of the raw MSW feed. The recently released Billion Ton Study[1] now includes MSW as a feedstock resource showing it to be one of the lowest cost and most widely distributed biomass resources available; however, acknowledging the concerns with the potential physical and chemical variability of an MSW based feedstock stream. The focus on MSW has also been driven by MSW being a resource with a clear, well understood and mature supply-chain. Treating MSW in the *BurCell*® System will make that source of biomass substantially more accessible. To further promote the use of this MSW derived biomass and beneficially exploit the use of the *BurCell*® System for processing of biomass for Bioenergy production facilities, more detailed lab data is now required

The treated materials organic fractions have preliminarily been shown to have higher concentrations of cellulose and other carbohydrates than raw MSW. Moreover, while raw MSW tends to be compositionally highly variable, the post *BurCell*® System material appears to be more homogenous. Processing raw MSW with the *BurCell*® System, besides aiding in the separation of non-organics, effectively pre-treats the material making it more amenable to bioprocesses through increased accessibility of the substrate. These processed organics are thus an inexpensive and easily processed source of fermentable cellulosic sugar. Preliminary conversion experiments have shown excellent recovery of glucose in mild operating conditions using moderate loadings of commercially available enzymes developed for the cellulosic ethanol industry. These same initial experiments have shown that these separated organics when compared to known literature data to be easier to process than pulped recycled paper. Compared to pulped recycled paper, *BurCell*® System separated organics require less pH adjustment and appear to provide better viscosity reduction and superior separation of hydrolysate from solids by filtration and/or centrifugation.

The goal of this work is to complete the quantitative characterization of the *BurCell*® System treated MSW materials, optimize enzyme loadings and develop a more accurate assessment of the simple sugars recovery potential. Attention also needs to be devoted to identify the potential for the formation of inhibitory compounds which would require characterization. This information will enable Cornerstone to develop a precise estimate of the value of *BurCell*® System processed organics as a source of fermentable sugar for bio-based processing and support accordingly its technical and business development activities. This information and data will also

[1] U. S. Department of Energy, Billion-Ton Report: Advancing domestic resources for a thriving bioeconomy, Volume 1: Economic availability of feedstocks. MH Langholtz, BJ Stokes, and LM Eaton (Leads). ORNL/TM-2016/160. Oak Ridge National Laboratory, Oak Ridge, TN 2016.

be incorporated into the Bioenergy Feedstock Library[2]; expanding the knowledge on MSW feedstocks for the bioenergy research community.

Materials and Methods

Samples

Five samples were created by Cornerstone to represent the compositional variability that would be observed in a MSW waste stream and treated with the *BurCell*® System. These samples were analyzed separately through chemical characterization and bench scale conversion assessments using both dilute-acid pretreatment and no pretreatment. A composite of these five materials was made for the bench scale conversion optimization experiments.

Chemical Characterization

Moisture of the samples was calculated after taking an initial weight of the frozen samples and then recording sample masses after thawing overnight at 40 °C and drying the samples at 40 °C, grinding the samples, and then further drying at 40 °C to around 10% moisture levels to maintain stability. The total solids measurement recorded during the first drying step in the compositional analysis procedure was used to calculate the initial moisture levels of the frozen samples. Compositional analysis to determine structural carbohydrates, lignin, extractives, and ash content was performed on material ground to 2 mm (Model 4 Wiley Mill, Thomas Scientific, Swedesboro, NJ) following the standard Laboratory Analytical Procedure for Compositional Analysis developed at the National Renewable Energy Laboratory (NREL).

Proximate, ultimate and calorimetric analyses of the biomass samples were performed on material ground to a homogenous powder using a household scale blender. Typically samples are ground using a knife mill to a specific particle size; however, deconstruction of these materials was difficult using the available analytical knife mills. A LECO Thermogravimetric Analyzer (TGA) 701 (St. Joseph, MI) was used for the standard proximate analysis based on determining moisture, volatiles, and ash for coal following ASTM D 5142-09. For the determination of moisture content the temperature is ramped to 107 °C at 6 °C/min under 10 lpm of UHP N_2. The sample was held at this temperature until a constant weight is reached. For the determination of volatiles, caps are added to the crucibles. The temperature was then ramped to 950 °C at 50 °C/min under 10 lpm of UHP N_2 and held for 9 minutes. For the determination of ash content, the instrument is allowed to cool to 600 °C and the caps are removed. The temperature was then ramped to 750 °C at 13 °C/min under 3.5 lpm of O_2 and held until a constant weight is reached for the sample. The difference between the volatile and ash measurement was considered fixed carbon. The volatiles measured on a dry basis are actually corrected using a calibration curve built on coal standards provided by LECO.

The determination of elemental C, H, and N was performed using a LECO TruSpec CHN (St. Joseph, MI). The analysis method used was one provided by LECO for analyzing flour and plant

[2] INL DOE Biomass Feedstock Library. bioenergy.inl.gov (accessed 10/9/2017).

tissues. The standards used for the CHN calibration was LECO provided EDTA. The carrier gas used for the combustion was UHP O_2 with a combustion temperature of 950 °C and afterburner temperature of 850 °C. The burn profile was 40 s high flow, 30 s medium flow, and 30 s high flow for complete combustion. All elemental carbon and hydrogen was oxidized and measured as CO_2 and H_2O using an infrared detectors. Resulting NO_x was reduced to N_2 using magnesium perchlorate anhydrone and analyzed with a UHP helium carrier gas by a thermal conductivity cell. Sulfur analysis was done with an add-on module for the CHN analyzer, LECO TruSpec S (St. Joseph, MI) following ASTM D4239-10. The sample was combusted under 3.5 lpm O_2 at 1350 °C and a minimum analysis time of 60 s. Oxygen content was determined by difference. Calorimetry was measured using a LECO AC600 (St. Joseph, MI) Isoperibolic system. The sample was combusted in a combustion vessel under 450 psi of UHP O_2. All corrections made for the final HHV calculations are based on the methods stated in the ASTM D 5865 using the moisture measured from the TGA and sulfur measured from ultimate analysis.

Elemental ash analysis was performed at Huffman Hazen Laboratories. The samples were dried overnight at 60 °C under air. Ash percentages were determined after slowly stage ashing the samples to 750 °C and holding this temperature for 8 hours under air. The resulting ash was fused and analyzed to avoid subsampling inhomogeneity from ash segregation. Elemental ash analyses are reported as calculated oxide equivalents on an ash weight basis.

Conversion Assessment

Conversion performance was determined using bench-scale enzymatic hydrolysis (EH) assays with and without dilute-acid pretreatment (DAPT). Laboratory-scale, DAPT was performed using a Dionex™ ASE™ 350 (Accelerated Solvent Extractor, ThermoFisher Scientific, Waltham, MA) according to Wolfrum et al[3] experiments were performed using 66 mL zirconium cells and a 10 % (*w/w*) solids loading with an acid-to-biomass loading of 0.08 g g^{-1}. Each cell was filled with 3.0 +/- 0.03 g biomass and 30 mL of 1 % sulfuric acid (*w/w*). Cells were subjected to a 7 min heating period followed by a 7 min static time with a reaction temperature of 160 °C. Then cells were purged for 200 s with nitrogen. The temperature was reduced to 100 °C and 100 to 150 mL of nanopure water was rinsed through the cell with a 200 s nitrogen gas purge. Aliquots of the rinsate were collected for determination of total and monomeric sugars using the same Four ASE cells were extracted for each sample. A minimum of three samples were then used for subsequent enzymatic hydrolysis.

Enzymatic hydrolysis of both the pretreated biomass and non-pretreated biomass was performed at 10% (w/w) solids loading and pH 4.8 at 50 °C, similar to the methods described by Wolfrum et al. Cellic® Ctec2 and Cellic® Htec2 enzyme complexes were provided courtesy of Novozymes® (Franklinton, NC). Protein content of enzyme complexes was measured using the bicinchoninic acid (BCA) assay using Micro™ BCA Protein Assay (ThermoFisher Scientific). Ctec2 and Htec2 had protein contents of 209 mg/mL and 207 mg/mL, respectively. Solid material approximately 1 g were added to 50-mL Erlenmeyer flasks on a dry weight basis. Sodium citrate buffer was added to achieve a biomass slurry at a concentration of 50 mM

[3] Wolfrum, E. J.; Ness, R. M.; Nagle, N. J.; Peterson, D. J.; Scarlata, C. J., A laboratory-scale pretreatment and hydrolysis assay for determination of reactivity in cellulosic biomass feedstocks. Biotechnology for Biofuels 2013, 6 (1), 162.

citrate, pH 4.8, 10% solids (dry weight) loading, and a final volume of 10 mL. The solids were enzymatically hydrolyzed using Ctec2 at a loading rate 40 mg/g biomass and Htec2 at 4 mg/g biomass. Sodium citrate buffer was supplemented with NaN3 to a final concentration of 0.02% in the biomass slurry in order to prevent microbial contamination. Flasks were incubated at 50 °C and 200 rpm for 72 hours in a Lab-Line incubator (Model 4628; Lab-Line Instruments, Inc.; Melrose Park, IL). Samples were removed, filtered, and diluted in water for HPLC analysis of glucose and xylose as previously described, at 6, 12, 24, 48, and 72 hours for the pretreated and non-pretreated samples. For optimization of enzyme loading, samples were removed and analyzed for glucose and xylose released at 72 hours. Enzyme and substrate blanks were prepared as controls for all experiments.

Glucose and xylose yields from dilute-acid pretreatment and enzymatic hydrolysis were calculated by dividing the sugar released by the initial sugar content in the biomass sample, including non-structural sugars. Reactivity for DAPT alone, DAPT and EH, and EH alone were calculated by the following equation:

$$Reactivity\ (\%) = \left(\frac{Glucose\ Released\ (g) + Xylose\ Released\ (g)}{Glucose\ Original\ (g) + Xylose\ Original\ (g)}\right) \times\ 100 \qquad (1)$$

where released sugars are those solubilized in dilute-acid pretreatment and/or enzymatic hydrolysis and original sugars are non-extractable (structural) and extractable glucan and xylan from compositional analysis.For the optimized conversion experiments using the composite of all five samples, no DAPT was used and the enzyme loadings were adjusted to loading rate of Ctec2 at 10, 20 and 40 mg/g biomass and Htec2 at 1, 2, and 4 mg/g biomass.

Results and Discussion

Chemical Characterization

The chemical characterization demonstrates the potential inherent variability of the feedstock produced from the *BurCell*® System (Tables 1-3). The total carbon is relatively consistent for all 5 samples, ranging from 46.2-48.6 % (Table 2). The components contributing to this carbon value vary greatly. Structural glucan varied from 34-43% and extractable glucan varied from 9-22 % contributing to an overall available carbohydrate variability of 63-68 % (Table 1). The inorganic fraction for the five samples also showed significant variability. All three methods for ash measurement; compositional analysis, proximate, elemental ash determination, agree that sample #5 had the highest ash content around 5 % total ash. The primary elemental component in samples #5 were from Al_2O_3, CaO, and SiO_2. Potassium was also found in higher levels for samples #1, #2, and #4. These components have identified specifications for biochemical conversion processes of <5 % ash and >59 % carbohydrates for feedstock quality determination.[4] Lignin, specifically acid-insoluble lignin, also significantly varied from 9-19 %. As

[4] Davis, R.; Tao, L.; Tan, E.; Biddy, M.; Beckham, G.; Scarlata, C.; Jacobson, J.; Cafferty, K.; Ross, J.; Lukas, J. Process design and economics for the conversion of lignocellulosic biomass to hydrocarbons: dilute-acid and enzymatic deconstruction of biomass to sugars and biological conversion of sugars to hydrocarbons; National Renewable Energy Laboratory (NREL), Golden, CO.: 2013.

lignin can be a source of recalcitrance for biochemical conversion processes, this variability should be considered for conversion efficiency. Overall these samples have much higher fraction of extractable components (20-38%) with a large fraction of available sugars located in the H_2O extractives fraction compared to standard herbaceous biomass resources used as biochemical conversion process feedstocks. These extractable sugars are accounted for and contribute to the conversion efficiency in these bench scale conversion tests; however, extractable sugars may be lost or converted to inhibitors during industrially relevant conversion processes greatly decreasing conversion efficiency.

Table 1. Compositional analysis for five MSW *BurCell*® treated samples; Ext.: extractable, AI: acid insoluble, AS: acid insoluble; n=2 for analytical replication of each sample; average and standard deviation (SD) are reported for the five samples.

Component (%)	#1	#2	#3	#4	#5	Avg.	SD
Initial Moisture	81.09	80.83	76.64	78.89	77.91	79.07	1.90
Whole Ash	3.81	2.28	3.19	2.57	5.01	3.37	1.09
Non-Ext Ash	1.37	0.87	1.65	1.00	5.14	2.01	1.78
Ext. Ash	2.44	1.41	1.54	1.58	0.50	1.49	0.69
Whole Protein	6.29	3.30	5.67	2.88	4.15	4.46	1.48
Non-Ext. Protein	2.72	1.55	3.09	0.88	1.94	2.04	0.89
Ext. Protein	3.57	1.75	2.58	1.99	2.21	2.42	0.71
H_2O Extractives	34.24	19.90	16.80	16.72	28.81	23.29	7.86
Ext. Glucan	21.55	11.99	8.96	8.68	17.56	13.75	5.64
Ext. Xylan	0.21	0.00	0.13	0.07	0.14	0.11	0.08
Ext. Galactan	0.84	0.51	0.32	0.43	0.74	0.57	0.22
Ext. Arabinan[a]	0.32	0.21	0.19	0.20	0.23	0.23	0.05
Water Extractives Others	8.88	5.77	5.65	5.77	9.64	7.14	1.95
Ethanol Extractives	3.56	3.64	5.09	3.52	6.22	4.41	1.21
Total Extractives	37.80	23.53	21.89	20.24	35.03	27.70	8.10
Lignin Total	11.77	20.17	15.83	17.21	10.90	15.18	3.85
AI Lignin	10.17	18.64	14.15	15.46	9.34	13.55	3.84
AS Lignin	1.60	1.53	1.68	1.75	1.56	1.62	0.09
Glucan	33.99	36.73	40.10	42.82	36.34	37.99	3.47
Xylan	4.93	5.09	5.82	6.48	5.09	5.48	0.66
Galactan	1.26	1.83	1.32	1.43	0.91	1.35	0.33
Arabinan[a]	5.05	8.30	6.21	7.32	3.75	6.13	1.80
Acetate	0.45	1.03	0.61	0.82	0.20	0.62	0.32
Whole Mass Closure	96.60	97.55	93.44	97.32	97.37	96.46	1.72

[a]Reported arabinan includes co-elution of any existing mannan.

Table 2. Proximate, ultimate, and calorimetric analysis for five MSW *BurCell*® treated samples; all values reported on a dry basis; n=3 for analytical replication unless otherwise noted; average and standard deviation (SD) are reported for the five samples.

Component	#1	#2	#3	#4	#5	Avg.	SD
Volatile (%)	81.60	81.29	82.46	82.28	82.36[a]	82.00	0.52
Ash (%)	3.50	2.44	3.13	2.54	5.72[a]	3.47	1.33
Fixed Carbon (%)	14.91	16.27	14.40	15.18	11.92[a]	14.53	1.61
Hydrogen (%)	6.16	6.00	6.28	6.14	6.12	6.14	0.10
Carbon (%)	46.84	48.32	48.57	48.25	46.19	47.63	1.05
Nitrogen (%)	1.37	0.72	1.23	0.63	0.90	0.97	0.32
Oxygen (%)	42.00	42.39	40.64	42.31	40.93	41.66	0.81
Sulfur (%)	0.14	0.13	0.15	0.13	0.13	0.14	0.01
HHV (BTU/lb)	8245	8584	8955	8538	8235	8511	296
LHV (BTU/lb)	6801	7140	7492	7106	6820	7072	282

[a]n=4.

Table 3. Elemental ash analysis for five MSW *BurCell*® treated samples; all values reported on a percent of total ash dry basis; n=2; average and standard deviation (SD) are reported for the five samples.

Component (%)	#1	#2	#3	#4	#5	Avg.	SD
Al_2O_3	5.70	12.58	11.91	5.48	12.63	9.66	3.73
CaO	18.64	12.17	21.12	17.36	32.44	20.35	7.51
Fe_2O_3	0.89	0.66	0.96	0.58	0.65	0.75	0.17
K_2O	22.33	23.47	8.76	18.05	8.04	16.13	7.34
MgO	5.06	4.04	3.30	3.84	2.72	3.79	0.88
MnO	0.08	0.14	0.10	0.17	0.05	0.11	0.05
Na_2O	12.16	11.71	17.09	15.61	8.08	12.93	3.54
P_2O_5	10.05	7.90	9.10	7.49	6.99	8.31	1.25
SiO_2	8.67	12.31	14.60	7.36	19.67	12.52	4.92
TiO_2	1.04	2.19	3.20	0.60	1.18	1.64	1.05
SO_3	6.99	6.23	6.10	5.31	3.69	5.66	1.25
Total Ash (% dry biomass)	3.26	2.48	3.06	2.55	5.33	3.34	1.16

Conversion Assessment

Like the composition there was variability between the samples between both conversion tests. The conversion test reactivity results (Table 4) and the visual material after enzymatic hydrolysis (Fig. 1) reflect this variability. Overall, the DAPT-EH tests resulted in higher sugar releases (Table 4). It should be noted that there was some inhibitor generation resulting from the DAPT-EH. For samples #1, #3, #4, and #5, 11, 11, 3, and 2 % of the total xylose released was converted to furfural, respectively. For the DAPT-EH samples #1 and #5 had the highest reactivity (>90% conversion efficiency) based on total available glucan and xylan and the lowest lignin concentrations. In contrast, sample #5 has the lowest reactivity when no DAPT was used, indicating that sample #5 could be more recalcitrant in some way compared to the other

samples or that the increase in ash played a role. Sample #4 had the same reactivity with and without DAPT and also had a significant amount more structural glucan than samples #1 and #5 (Table 1). Sample #2 could not be tested for conversion as there was not enough liquid available to sample during EH; however, based on composition and visual comparison (Fig. 1), sample #2 probably has similar reactivity to sample #4.

Table 4. Dilute-acid pretreatment (DAPT) and enzymatic hydrolysis (EH) and EH alone glucose release and xylose release conversion assessments for five MSW BurCell® treated samples; all values reported on a grams sugar released per gram sugar available; n=3 for analytical replication unless otherwise noted; averages (Avg.) and standard deviations (SD) are reported for the five samples.

Conversion (g/g)	#1	#2	#3	#4[a]	#5	Avg.	SD
DAPT/EH[b]							
DAPT Glucose release	0.46	0.35	0.30	0.27	0.48	0.37	0.10
DAPT Xylose release	0.61	0.61	0.54	0.46	0.35	0.51	0.11
DAPT Reactivity	0.47	0.38	0.33	0.29	0.47	0.39	0.08
EH Glucose release	0.47	[c]	0.51	0.35	0.42	0.43[d]	0.07[d]
EH Xylose release	0.34	[c]	0.24	0.25	0.55	0.35[d]	0.14[d]
DAPT/EH Reactivity	0.93	[c]	0.80	0.63	0.90	0.82[d]	0.14[d]
EH[e]							
EH Glucose release	0.81	0.61	0.71	0.64	0.52	0.66	0.11
EH Xylose release	0.59	0.41	0.52	0.54	0.43	0.50	0.08
EH Reactivity	0.79	0.60	0.69	0.63	0.51	0.64	0.10

[a]n=3 for DAPT/EH conversion results
[b]n=4 for all 5 samples unless noted otherwise
[c]Not enough liquid to sample for EH
[d]Batch #2 not included in calculation
[e]n=3 for all 5 samples

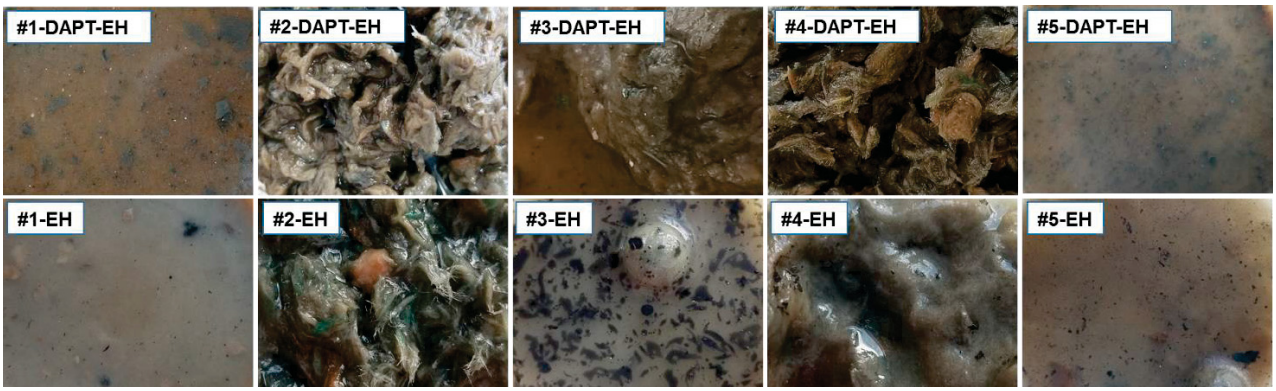

Figure 1. Photos of the five samples after enzymatic hydrolysis (EH) with dilute-acid pretreatment (top) and without (bottom).

Along, with final reactivity for both the DAPT-EH and EH experiments, reactivity was measured at various time points (Fig. 2). These can help to optimize the reaction time and determine if the trends in the sugar releases are consistent throughout the enzymatic hydrolysis reaction. Note the decrease in reactivity for sample #1 in the DAPT-EH data at 12 hr and the EH alone reaction at 48 hr are anomalies due to general analytical error throughout the process. The trends between the different samples and the DAPT-EH and EH experiments, in general, do not differ over the different time points.

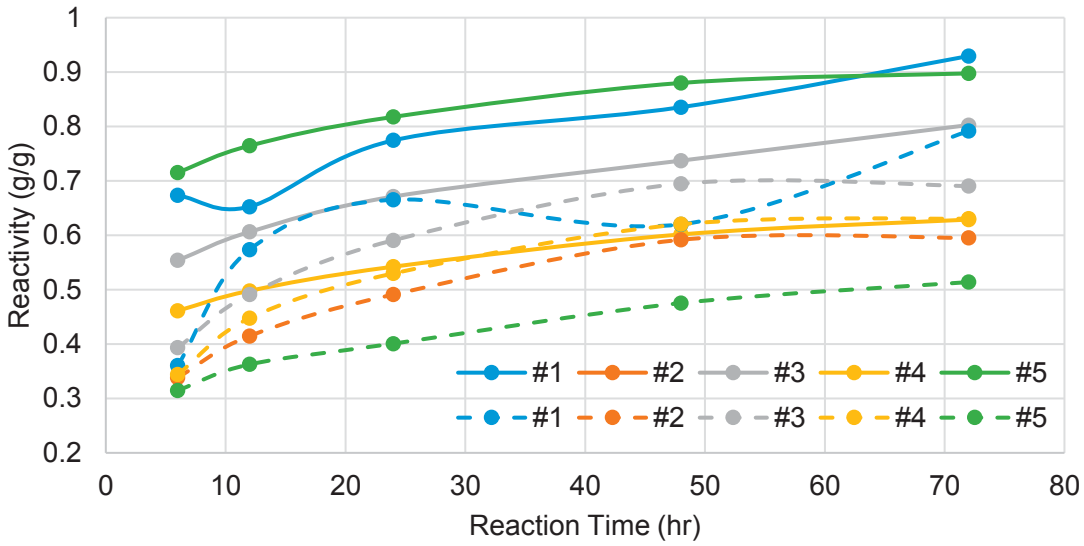

Figure 2. Reactivity for both dilute-acid pretreatment (DAPT) and enzymatic hydrolysis (EH) (—) and EH alone at 6, 12, 24, 48, and 72 hours (− −) on a grams sugar released per grams sugar available.

Conversion Optimization

The purpose of these experiments was to determine the impact of adjusting the enzyme loadings. No DAPT was used for these experiments; however based on the previous experiments it can be assumed that the overall reactivity would have increased. A representative sample using all five materials was created for these tests assuming that these five samples would be represented equally within an actual processes batch. As 40 mg/g biomass Ctec2 and 4 mg/g biomass is typically considered to be a high enzyme concentration, lower concentrations were assessed of 10 and 20 mg/g of Ctec2 and 1 and 2 mg/g of Htec2 were considered. Overall the lower enzyme loadings did negatively impact the reactivity and releases for both glucose and xylose. This data indicate that the trade-offs between enzyme cost and conversion efficiencies need to be assessed.

Table 5. Enzymatic hydrolysis (EH) glucose release and xylose release conversion assessments for composites of five MSW BurCell® treated samples using loading rate of Ctec2 at 10, 20 and 40 mg/g biomass and Htec2 at 1, 2, and 4 mg/g biomass; all values reported on a grams sugar released per gram sugar available; n=3 for analytical replication unless otherwise noted.

Conversion (g/g)	Ctec2 10: Htec2 1	Ctec2 20: Htec2 2	Ctec2 40: Htec2 4
EH			
EH Glucose release	0.46	0.58	0.65
EH Xylose release	0.37	0.46	0.50
Reactivity	0.46	0.57	0.64

Conclusion

Based on total carbon, the five samples were all very similar however the organic components between the samples varied significantly; specifically the lignin and carbohydrate contents. This variation in composition and physical format resulted in variability in the amount of sugars that were released following a DAPT-EH treatment and EH treatment; ranging from 51-93% conversion efficiency. The simulated treatment based on the *BurCell*® System did not completely negate the need for additional pretreatment steps prior to enzymatic hydrolysis in order to maximize the amount of sugars released. Additionally, the reactivity of these samples did decrease with decreased enzyme concentrations; however, this decrease was not proportional. When the enzyme concentrations was decreased by 50 % the overall reactivity only decreased 7 %.