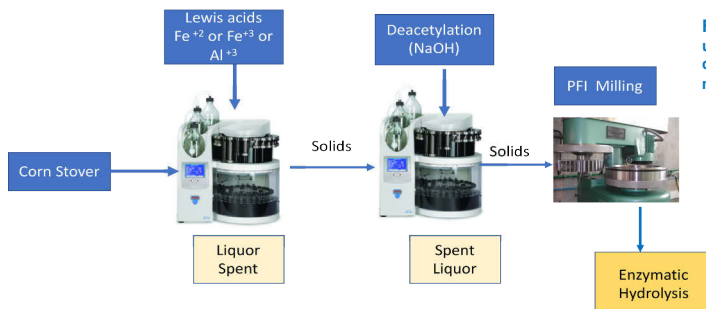


Enhanced bioconversion in herbaceous feedstocks from impregnation of co-catalytic Lewis acid and deacetylation and mechanical refining

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Abstract

Improvements in conversion processes are needed to further reduce process complexity, biorefinery capital and operating (CAPEX/OPEX) costs, lower enzyme usage. Previous work has demonstrated that deacetylation and mechanical refining (DMR) process effectively deconstructs herbaceous biomass while producing highly digestible lignocellulosic slurries, with high titers of monomeric sugars, at reduced enzyme loadings (Poster 553 Chen et al.). We evaluated a co-catalytic approach using three Lewis acids, Fe^{2+} , Fe^{3+} , and Al^{3+} , in sulfate form, at 1 mM, 5 mM and 10 mM concentrations. Lewis acids were impregnated into corn stover, followed by deacetylation with NaOH, and mechanically refined, prior to enzymatic hydrolysis (EH). Lower concentrations of Lewis acid positively impacted EH yield, increasing concentration lowered EH yield. Likewise Lewis acid treatment was more effective when combined with deacetylation than with deacetylation or Lewis acid treatment, alone. Comparing the effectiveness of the three Lewis acids identified ferrous iron more effective than ferric or aluminum ($Fe^{2+} > Fe^{3+} > Al^{3+}$) sulfate. Enzymatic hydrolysis sugar (glucose and xylose) concentration was higher 8% and 13%, respectively, in Fe^{2+} impregnated corn stover compared to the control. Refining energy required for PFI milling was reduced by ~25% with Fe^{2+} impregnation. However, treatment with Fenton treatment of the Fe^{2+} impregnated, deacetylated and PFI milled corn stover resulted in lower yields, for monomeric glucose and xylose due to non-specific degradation of carbohydrates and lignin.



Process Diagram

Figure 1. Diagram of biomass pre-impregnation using Lewis acids (sulfate cation), followed by deacetylation @70kg NaOH/DWT, followed by PFI milling and enzymatic hydrolysis.

Key variables:

- Type of Lewis acid, Fe, Fe, and Al
- Concentration, 1mM, 5mM or 10 mM
- ASE 350 reaction time and temperature
- Hydrogen peroxide loading
- Enzyme loading

Take Home Message

- Lewis acid impregnation using Fe^{2+} was more effective than Al^{3+} or Fe^{3+} at increasing enzymatic hydrolysis yields. Highest glucose yield from EH was achieved at 1mM to 1.5 mM Fe^{2+} impregnation, from batch experiments.
- Lewis acid impregnation using 1mM Fe^{2+} was more effective at reducing PFI refining energy (~25%) compared to control.
- Increasing scale for the Lewis acid impregnation and deacetylation did not increase EH performance compared to control.
- Over-treatment with Fenton chemistry w/w/o Fe^{2+} impregnation may decrease EH digestibility.
- Fenton treatment removed significant amounts of cellulose and lignin resulting in higher content but lower mass of final sample.

Future Work

- Using imaging techniques (Prussian Blue) to identify location and extent of Fe^{2+} in biomass tissues, prior to and post deacetylation.
- Additional investigation into scaling the process is needed to address differences between high and low solids results
- Evaluate ozonolysis of DMR treated biomass to enhance EH yield of glucose and xylose. Ozonolysis provides more selectivity and reduces non-specific oxidation of carbohydrates.

Materials and Methods

Small scale impregnation and deacetylation

- ASE350 accelerated solvent extractor (Dionex, Sunnyvale, CA, USA) using corn stover milled to 2 mm, then treated, first with a co-catalyst salt (Table 1) at a volume of 40 mL at 80 C for 30 minutes, drained then with sodium hydroxide (170 mM) at a volume of 40 mL at 92 C for 60 minutes.

PFI milling

- Approximately 30 g, at 20 wt% total solids, of processed corn stover was placed in the PFI mill. The shearing and compression forces produced by the impacts of the rotor bar cause intra-fiber bond breaking, external fibrillation and fiber cutting. All samples were refined at 6000 revolutions. Power consumption resulting from refining was measured for each experimental condition

Low solids enzymatic hydrolysis

- Enzymatic digestions of washed refined residues from the deacetylation/mechanical refining experiments were performed in 125 ml Erlenmeyer shake flasks at 1% cellulose solids loading (approximately 2 wt% solids loadings), 50° C, and 130 rpm. Cellulase and hemicellulase enzyme preparation were added at ~15 mg protein/g cellulose (total) assuming a cellulose content of 50%.

Large scale impregnation and deacetylation

- Prior to deacetylation corn stover was impregnated in the with 1.5 mM $FeSO_4 \cdot 7H_2O$ at 8% solids (w/w) loading for 2 hr, at 60°C in the 1900-L paddle mixer. After impregnation the liquor was drained, prior to deacetylation (Poster 553). Deacetylation was performed by adding 125kg corn stover at 93%(w/w) solids (116 bone dry kg) was added to the paddle mixer along with a dilute (0.2 M) sodium hydroxide solution to produce a 9:1 liquid to solids (10 wt% total insoluble solids) slurry. The slurry was heated to 92° C and held for 2 hours, and then the black liquor could drain overnight. Water was added; the solids were then mixed for 1 hour and drained from the mixer through the same screens. Solids were dewatered using a continuous screw press

Chemical compositional analysis

- Liquors resulting from DMR, and enzymatic saccharification liquors, were analyzed using HPLC according to standard NREL laboratory analytical procedures (LAPs) (Sutler et al., 2008a). Sugar yields from high solids enzymatic hydrolysis were calculated using the equations developed by (Zhu et al., 2011).

Biomass treatment using Fenton chemistry

- The hydrogen peroxide loadings are 0.6g/g of dry biomass, with a $FeSO_4 \cdot 7H_2O$ catalyst loading of 33 mg/g of dry biomass. The samples were pre-treated with 6.0 mM $FeSO_4 \cdot 7H_2O$ prior to deacetylation. 50g/dwt biomass was loaded into a batch-type reactor, a 4 L (1 L working volume) ZipperClave® reactor (Autoclave Engineers). The internal canister was heated to 70°C. Mixing is achieved in the ZipperClave® reactor using a modified anchor-type impeller with customized lifting wedges. Biomass was first impregnated with 6 mM $FeSO_4 \cdot 7H_2O$ solution and heated to 70C for 1hr. 300mL of a H_2O_2 solution was pumped into the ZipperClave®, followed by chase of 100mL of DI water. Final H_2O_2 concentration was 3% with a 20:1 ratio of H_2O_2 to g of solid. Reaction time was 15 minutes, after completion the slurry was then recovered, the solids and liquors were separated. Solids were washed prior to compositional analysis.



Figure 2. DMR pretreated corn stover (Left) versus DMR pretreated corn stover following treatment using Fenton reactions (Middle). Right: condensate collected after reaction.

Results

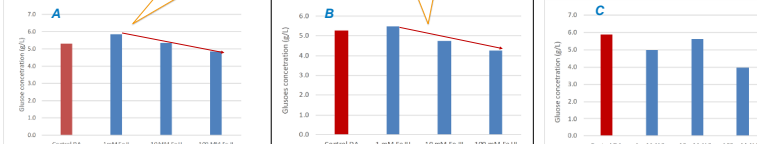


Figure 3. Enzymatic hydrolysis glucose sugar release following deacetylation/mechanical refining and Fe^{2+} (A), Fe^{3+} (B) and Al^{3+} co-catalyst impregnation prior to deacetylation/mechanical refining of corn stover.

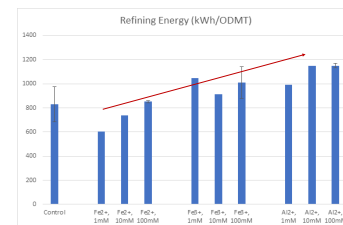


Figure 4. Effects of Lewis acid co-catalyst additions on mechanical refining energy of deacetylated corn stover feedstocks.

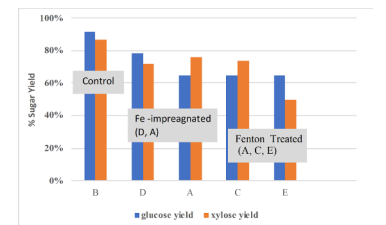


Figure 5. Monomeric glucose and xylose yields from low solids (2%) enzymatic hydrolysis of pilot-scale iron pre-impregnated, deacetylated and PFI refined samples

Table 1. Composition of Fenton treated (A, C, E) and Non-treated (B, D) corn stover samples

Sample ID	Ash	Lignin	Glucan	Xylan	Galactan	Arabinan	Acetate	Total
A	2.53	11.90	71.49	9.49	0.33	0.44	0.18	96.36
B	1.48	9.92	58.93	24.75	0.91	2.72	0.13	98.83
C	1.91	15.93	67.92	10.73	0.00	0.32	0.20	97.02
D	1.17	12.59	57.40	25.24	0.85	2.65	0.16	100.07
E	2.91	16.90	60.74	16.87	0.34	1.31	0.45	99.52