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High-Solids Enzymatic Saccharification Screening Method for Lignocellulosic Biomass

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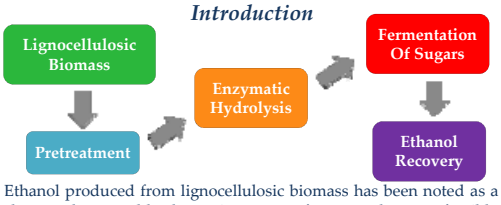
Abstract

The ability to screen new biomass pretreatments and advanced enzyme systems at process-relevant conditions is key to developing economically viable lignocellulosic ethanol. While much research is being invested in developing pretreatment technologies and enzyme systems that will more efficiently convert cellulosic biomass to sugars, the current standard reactor vessel, a shake flask, that is used for screening enzymatic saccharification of cellulosic biomass is inadequate at high-solids conditions. Shake flasks do not provide adequate mixing at high solids conditions. In this work, **a roller bottle reactor was identified as a small-scale high-solids saccharification reaction vessel**, and a method was developed for use in **screening both pretreated biomass and enzyme systems at process-relevant conditions**. This new method addresses mixing issues observed in high-solids saccharifications. In addition, **yield calculations from sugar concentrations on a mass basis were used** to account for the two-phase nature of the saccharification slurry, which eliminates discontinuities in comparing high-solids to low-solids saccharifications that occur when using concentrations on a volume basis. The roller bottle reactors out-performed the shake flasks by 5% for an initial insoluble solids loading of 15% and 140% for an initial soluble solids loading of 30%. The reactor system and method was compared at bench and floor scales and determined to be scalable for initial insoluble solids loading in the range of 15% to 30%. Pretreatment and enzyme screening results indicate that mid severity pretreated biomass is more digestible than the low and high severity biomass and GC220 is a superior enzyme to Spezyme CP.

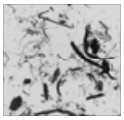
Introduction



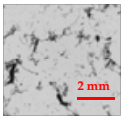
Ethanol produced from lignocellulosic biomass has been noted as a clean and renewable domestic source of energy that can feasibly displace a significant fraction of petroleum usage in the United States.[1] However, significant challenges exist with breaking down biomass to fermentable sugars. Research indicates that a chemical pretreatment followed by enzymatic hydrolysis increases the overall efficiency of saccharification.[2,3] One promising approach to improving process economics is to increase biomass concentration during pretreatment and enzymatic hydrolysis, thereby resulting in higher product concentrations. In order to exploit the synergism of pretreatment and enzymatic hydrolysis at process-relevant conditions, it is necessary to be able to effectively screen pretreated biomass and enzyme preparations at high-solids loadings in small-scale reactors.

Biomass Substrate – Dilute Acid Pretreatment

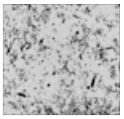
Steam Digester: 4-L steam explosion batch reactor
Sunds Reactor : Continuous 1 ton/day pilot-scale vertical reactor



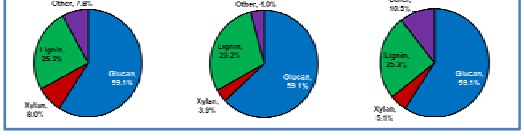
Low Severity
- 33A14 Corn Stover
- ¼ inch milled
- Steam Digester
- 7.25 mg H_2SO_4/ g dry biomass
- 150°C – 30 min
- Composition (wt/wt):



Mid Severity
- 33A14 Corn Stover
- ¼ inch milled
- Steam Digester
- 7.25 mg H_2SO_4/ g dry biomass
- 170°C – 30 min
- Composition (wt/wt):



High Severity
- 34M95 Corn Stover
- ¾ inch milled
- Sunds
- 48 mg H_2SO_4/ g dry biomass
- 190°C – 1 min
- Composition (wt/wt):



Enzymatic Saccharification

Roller bottle reactors (RBR) are loaded by mass to half of the volume of the vessel, assuming a density of 1g/ml. RBRs are charged with washed pretreated biomass, buffer and anti-microbial agent, and then brought to temperature while mixing before adding enzyme. Enzyme is distributed on the surface of the biomass slurry across the length of the vessel. Mixing is achieved through gravitational tumbling by rotating the RBRs horizontally on a Wheaton Mini Roller Apparatus. Temperature control is achieved by housing the roller apparatus in a general purpose floor-standing incubator.

Experimental Conditions:
- 15, 20, or 30% insoluble solids (IS) loading (mass basis)
- 5, 10, 15, 20, or 30 mg protein/ g cellulose enzyme loading
- Genencor GC 220 cellulase (Lot # 4900759448, 220 mg protein/ mL) or Genencor Spezyme CP cellulase (Lot# 301-05021-011, 134 mg protein/ mL)
- pH – 4.8
- 10 µg/mL tetracycline
- Temperature - 48°C
- 2 – 4 rpm
- 7 days

Yield Calculations

- Concentrations are converted from g/L to g/g slurry

$$f_i = \frac{\Delta c_i}{\rho_l}(1 - f_{is})$$

f_i: mass fraction [g *i*/g slurry]
c_i: liquid concentration [g *i*/L liquid]
ρ_l: liquid density [g/L]
f_{is}: [g insoluble solids/g slurry]

- Yields are calculated based on maximum theoretical yield

General Conversion Equation

$$\xi = \frac{\sum_{i=1}^{N} r_{j/i}\Delta f_i}{f_{is,0}\sum_{j=1}^{M} x_{j,0}}$$

$r_{j/i}$: MW ratio of polysaccharide component *j* to mono- or oligosaccharide *i*
Δf_i: increase in *i* over time
x_j: mass fraction [g *j*/g insoluble solids]

Cellulose Conversion

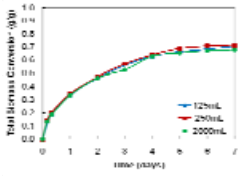
$$\xi_C = \frac{r_{G/g}\Delta f_g + r_{G/cb}\Delta f_{cb}}{f_{is,0}x_{G,0}}$$

Total Biomass Conversion

$$\xi_{TB} = \frac{r_{G/g}\Delta f_g + r_{G/cb}\Delta f_{cb} + r_{X/x}\Delta f_x}{f_{is,0}(x_{G,0} + x_{X,0})}$$

G: glucan
g: glucose
cb: cellobiose
X: xylan
x: xylose
0: initial condition

Scalability



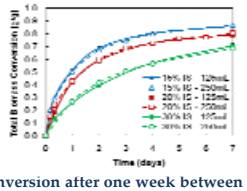
Conditions:
- 20% IS loading high severity PCS
- Spezyme CP (20 mg/g cellulose)
- Roller Bottles – Size / Rotation

Size	Rotation
125 mL	4 rpm
250 mL	2 rpm
2000 mL	2-20 rpm

- **Enzymatic saccharification reactions do not appreciably differ for conditions tested**
- **All conditions reached about 70% of theoretical total biomass conversion after one week**

Conditions:
- High severity PCS
- GC220 (20 mg/g cellulose)
- Roller Bottles – Size / Rotation

Size	Rotation
125 mL	4 rpm
250 mL	2 rpm



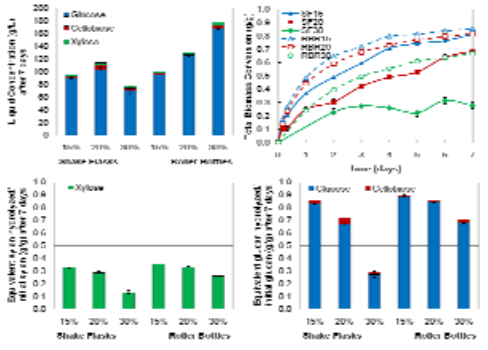
- **No significant difference in conversion after one week between roller bottle sizes (125 mL to 250 mL) at insoluble solids loadings ranging from 15% to 30%.**

Note: Uncertainty error bars of 95% confidence interval are included but are not larger than the data point size

Shake Flasks VS Roller Bottles

Conditions:
- High severity PCS
- GC220 (20 mg/g cellulose)
- Shake Flasks – 130 rpm
- Roller Bottles – 2 rpm

Observations:
- No material movement in 20% or 30% IS Shake Flasks while shaking
- 30% IS Shake Flask never fully wet
- 30% IS Roller Bottle fully wet in 4 hr



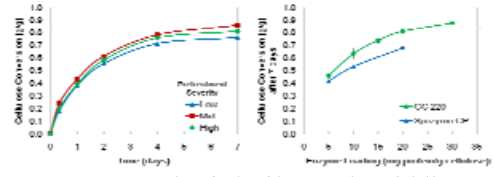
Note: Constant uncertainty was assumed for each experimental condition over the entire enzymatic saccharification process. Error bars are for a 95% confidence interval.

- **Roller Bottles give 2.4 times the total biomass conversion as Shake Flasks at 30% insoluble solids loadings**
- **Shake Flasks exhibit lesser efficiency and greater variability in converting biomass at all insoluble solids loadings**
- **Glucose concentrations were observed as high as 170 g/L at 30% insoluble solids loading**

Pretreatment and Enzyme Screening

Conditions:
- 20% IS loading
- GC220 (20 mg/g cellulose)
- Roller Bottles – 125 mL; 4 rpm

Conditions:
- 20% IS loading high severity PCS
- GC220 and Spezyme CP
- Roller Bottles – 125 mL; 4 rpm



Note: Uncertainty error bars of 95% confidence interval are included but typically are not larger than the data point size.

- **Mid severity pretreated corn stover has the highest digestibility of the three pretreatment severities for given conditions tested**
- **GC220 out-performs Spezyme CP. At all loadings tested After 7 days of hydrolysis with loadings of 20% IS and 20 mg/g cellulose enzyme, GC220 converts 81% cellulose while Spezyme CP converts 68%**

Summary

- **Roller Bottles perform significantly better than traditional Shake Flasks as enzymatic saccharification pretreatment and enzyme screening reaction vessels, likely due to more effective mixing by horizontal tumbling than shaking**
- **Roller Bottle vessels are scalable between bench and floor scales and for insoluble solids loadings between 15% and 30%**
- **High-solids enzymatic saccharification screening method can be applied to a variety of biomass feedstocks and enzymes**
- **Using this high-solids enzymatic saccharification screening method, the mid severity pretreatment and GC220 enzyme were identified as top performers of the conditions tested**

References

1. Jørgensen et al. (2007) *Biofuels, Bioprod Biorefin*, 1(2):119-134
2. Mosier et al. (2005) *Bioresour Technol*, 96(6), 673-686
3. Rosgaard et al. (2007) *Biochem Biotechnol*, 143(3), 284-296

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