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# On-farm pretreatment technologies for improving enzymatic degradability of cellulose and hemicellulose present in perennial grass

Matthew F. Digman[1,2], Kevin J. Shinners[1], Bruce S. Dien[3], Ron D. Hatfield[2], Richard E. Muck[1,2], Xin-Liang Li[3], and Paul J. Weimer[2]

[1]Department of Biological Systems Engineering University of Wisconsin - Madison, [2]United States Dairy Forage Research Center (USDA-ARS), [3]National Center for Agricultural Utilization Research (USDA, ARS)

## Introduction

Recently, wet storage methods have been proposed for feedstock preservation and on-farm storage of perennial grass and corn stover biomass. The advantages over a dry storage system include:

- ✓ reduced harvest costs
- ✓ less weather risk at harvest
- ✓ greater biomass yields
- ✓ improved feedstock susceptibility to enzymatic hydrolysis

Bunk storage of forage

We believe that wet storage systems may also present a unique opportunity to add value to the feedstock through chemical or biological pretreatment. In-storage pretreatments at ambient temperature and pressure but prolonged reaction times may lower pretreatment costs and provide better return for farmers.

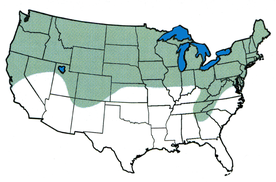
Our research investigates the ability of on-farm pretreatments including:

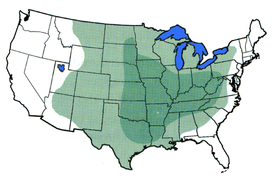
- dilute acid ($H_2SO_4$)
- alkali ($CaOH_2$)
- ozonolysis ($O_3$)
- novel enzymes (feruloyl esterase, xylanase)

to improve enzymatic degradability of cellulose and hemicelluloses in biomass at the biorefinery.

## Method

I. Feedstocks:


Reed Canarygrass (*Phalaris arundinacea*)†


Switchgrass (*Pancium virgaum*)†

II. Substrate composition:

- Cell wall isolated by Uppsala Total Dietary Fiber Method
- Neutral sugar analysis as alditol acetates after two step acid hydrolysis
- Sugars quantified by GLC-FID

III. Storage:

- Anaerobic at ~ 22°C
- 30 day duration

WECK Canning Jars, "Mini-silos"

IV. Ethanol Yield (SSF):

- Fermentation substrate undried and unground (4 mm)
- Genencor GC220 cellulase (5 FPU/g DM)
- Novozyme Novo188 β-glucosidase (15 U/g DM)
- *Sacchromyces cerevisiae* D5A
- 72 hr fermentation
- Cellulose degradation assayed by measuring ethanol via HPLC

† Barnes, R., C. Nelson, M. Collins, and K. Moore. 2003. Forages an introduction to grassland agriculture sixth edition. USA: Iowa State Press, Ames, IA.

## Results | Acid & Lime Pretreatment

: Experiment Design

| OFAT Design | |
|---|---|
| Substrate | Reed Canarygrass (RCG)<br>Switchgrass (SWG) |
| Chemical | Acid: $H_2SO_4$ (3, 9 %DM)<br>Alkali: $CaOH_2$ (5, 15 %DM) |

Results:

Pile storage of forage

| Crop | Treatment | Conversion Efficiency: Cellulose η† | Conversion Efficiency: Xylose η† |
|---|---|---|---|
| RCG | Control | 33% | 6% |
| | Acid Low | 54% | 13% |
| | Acid High | 67% | 26% |
| | Alkali Low | 54% | 23% |
| | Alkali High | 79% | 67% |
| SWG | Control | 15% | 9% |
| | Acid Low | 25% | 16% |
| | Acid High | 29% | 23% |
| | Alkali Low | 24% | 23% |
| | Alkali High | 42% | 77% |

†LSD = 4.1% & 2.5% at α = .05 for cellulose and xylose respectively

## Results | Ozonolysis

: Experiment Design

| OFAT Design | |
|---|---|
| Substrate | Reed Canarygrass (RCG)<br>Switchgrass (SWG) |
| Duration | 1, 2, & 5 hours<br>(5 %wt/wt concentration) |

Results:

Treated (left), Untreated (right)

| Crop | Treatment | Conversion Efficiency: Cellulose η† | Conversion Efficiency: Xylose η† |
|---|---|---|---|
| RCG | Control | 42% | 10% |
| | 1 hour | 67% | 30% |
| | 2 hours | 71% | 41% |
| | 5 hours | 59% | 41% |
| SWG | Control | 16% | 11% |
| | 1 hour | 27% | 21% |
| | 2 hours | 32% | 33% |
| | 5 hours | 41% | 53% |

†LSD = 3.3% & 3.0% at α = .05 for cellulose and xylose respectively

## Results | Novel Enzymes

: Experiment Design

| $2^4$ Factorial Design | |
|---|---|
| Substrate | Reed Canarygrass (RCG)<br>Switchgrass (SWG) |
| Feruloyl esterase | 100, 10 U/kg DM |
| Xylanase family 10 | 1000, 0 U/kg DM |
| Xylanase family 11 | 5000, 0 U/kg DM |

Application of enzyme

Results:

| | FAE | Family 10 | Conversion η |
|---|---|---|---|
| RCG | 100 | 0 | 38.1% |
| | 100 | 1000 | 40.2% |
| | 10 | 0 | 40.2% |
| | 10 | 1000 | 37.3% |
| SWG | 100 | 0 | 19.0% |
| | 100 | 1000 | 18.8% |
| | 10 | 0 | 19.0% |
| | 10 | 1000 | 18.9% |

†LSD = 2.7% at α = .05

## Conclusion

- ✓ Conversion efficiencies of nearly 80% for cellulose and hemicellulose realized.
- ✓ Sulfuric acid and lime pretreatment technologies look promising when considering conversion rates and ease of integration into current storage systems.
- ✓ Ozonolysis results look promising, but integration into current storage systems will be challenging.
- ✓ Enzyme addition (xylanases or feruloyl esterase) at ensiling only marginally improved conversion efficiency.

## Future Work

- ✓ Develop response surface to optimize pretreatment conditions
- ✓ Perform pretreatment at farm scale to consider scale-up issues
- ✓ Consider pretreatment robustness given substrate
- ✓ Evaluate ozonolysis given low concentration but long duration

Direct harvest of switchgrass

## Acknowledgements

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BIOLOGICAL SYSTEMS ENGINEERING
University of Wisconsin - Madison
College of Agricultural and Life Sciences

**United States Dairy Forage Research Center**
USDA-ARS
1925 Linden Drive West
Madison, WI 53706

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