National Renewable Energy Laboratory

Microbial Pretreatment of Biomass: Potential for Reducing the Severity of Thermochemical Biomass Pretreatment

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Abstract

Typical pretreatment requires high energy (steam and electricity) and corrosion resistant, high-pressure reactors. Our literature assessment suggests that fungal pretreatment could potentially lower the severity requirements of acid, temperature and time. These reductions in severity are also expected to result in less biomass degradation and consequently lower inhibitors. Furthermore, potential advantages of fungal pretreatment of agricultural residues, such as corn stover, are suggested by its effectiveness in improving the cellulose digestibility of many types of forage fiber and agricultural wastes.

Preliminary tests show a 3 to 5-fold improvement in enzymatic cellulose digestibility of corn stover after pretreatment with *Cyathus stercoreus*; and a ten to 100-fold reduction in shear force needed to obtain the same shear rate of 3.2 to 7 rev/sec, respectively, after pretreatment with *Phanerochaete chrysosporium.*

Introduction

- Effective pretreatment is required in enzyme-based lignocellulosic ethanol processes to significantly improve the cellulose digestibility.
- The cost of dilute acid pretreatment has been identified as the second highest (18% of total production cost) after feedstock cost (33%) for ethanol production from corn stover[1].
- The major pretreatment cost components include:
  - Capital recovery charge
  - Chemicals and process energy (steam and electricity)
- Literature review indicates that microbial pretreatments reduce the refining energy required in mechanical pulping of wood chips and improve the cellulose digestibility of straws and grasses.

1 "Enzyme Sugar-Ethanol Platform Project", National Renewable Energy Laboratory, Golden, CO., U.S. (2002) http://www.ott.doe.gov/biofuels/esp_background.html.

Literature Review

Fungal Pretreatment of Hardwood and Softwood

- 23% reduction in pulp refining energy required by incubating spruce and pine chips for two weeks with the white-rot fungus *Phanerochaete chrysosporium* at 35 - 40°C [2,3].

- 47% reduction of refining energy required in mechanical pulping after a four-week treatment with *Ceriporiopsis subvermispora sp.* on aspen wood chips and a 30% reduction in extractable resin compared to controls. Energy savings of 37% on Loblolly pine were obtained. The weight losses on each were about 6% on aspen and 5% for Loblolly pine after 4 weeks of fungal treatment [4,5].

4 Messner, K. and Srebotnik, E. (1994) *FEMS Microbiological Reviews*, **13**, 351- 364.

5 Akhtar, M., et al. (1992) "The white-rot fungus *Ceriporiopsis subvermispora* saves electrical energy and improves strength properties during biomechanical pulping of wood", Proceedings of the 5th International Conference on Biotechnology in the Pulp and Paper Industry, M. Kuwahara and M. Shimada, Ed., 3-8, Uni Publishers Co., Ltd., Tokyo.

Literature Review

- Treating coarse mechanical pulp with a white-rot fungus, *IZU-154* for 7 days reduced refining energy of beech pulp by 66%. The lignin and total weight losses were 17.5% and 4.3% respectively [6].

- Pretreatment of beech wood meal by *Phanerochaete chrysosporium* at 37°C for 28 days followed by steam explosion at 215°C for 6.5 min improves the enzymatic cellulose digestibility, compared to steam explosion alone, by as much as 100%. However, 17% of the holocellulose degraded during fungal pretreatment [7].

6 Kashino, Y. et al. (1993) *Tappi Journal*, **76**, (#12), 167-171.

7 Sawada, T. et al. (1995) *Biotechnology & Bioengineering*, **48**, No. 2, 719-724.

Literature Review

Fungal Pretreatment of Agricultural Residues

- 15-day treatment of maize and rice straw by *Cyathus stercoreus* improved the *in vitro* cellulose digestibility by 37% and 45%.
  The dry matter loss was only about 3% [8].
  No direct correlation between lignin degradation and improved digestibility.
  In comparison, *Phanerochaete chrysosporium* degraded both hemi-cellulose and cellulose.

- 72-h treatment with *Ceriporiopsis subvermispora fp*-90031 improved *in vitro* ruminal digestion of Bermuda grass by 80% [9].

8 Karunanandaa, K. et al. (1992) *J. Sci. Food Agric.,* **60**, 105-112.

9 Akin, D. E.. Et al. (1993) *Applied and Environmental Microbiology*, **59**, No. 12, 4274-4282.

Materials & Methods

Fungal strains selected for preliminary testing:

- *Cyathus stercoreus* NRRL 6473 (delignification of maize)
- *Phanerochaete chrysosporium* BKM-F-1767 (delignification)

Culturing and Preservation:

- The strains were grown on Potato Dextrose Agar
- Cultures were preserved in 25% glycerol + Tween 80 and stored at –70^{o}C

Corn Stover Preparation:

- Stover was washed, air-dried to 30% moisture, frozen-milled through 2-mm screen, kept frozen until use.

Materials & Methods

Fungal Pretreatment of Corn Stover (Shake Flasks):

- Grew *Cyathus stercoreus* and *Phanerochaete chrysosporium* on Potato Dextrose Agar. Homogenized in 0.05M phosphate buffer pH 6 (1:1 agar : buffer solution).

- Added 5 mL of culture suspension and 20 µL of 12 mg/L tetracycline-HCl (in 70% EtOH) to 10 g (30% moisture) of sterilized corn stover in 250-mL Erlenmeyer flasks (with Morton closures). Sterilized water added to bring the moisture content to about 88%.

- Incubated the flasks at 27^{o}C, 150 rpm for 29 days. No bacterial contamination observed.

- Added sterilized water every 3 days to maintain weight of flasks.

Materials & Methods

Fungal Pretreatment (Roller Bottles):

- 100 g of corn stover in 2-L plastic bottles
- Inoculated with *Phanerochaete* culture suspension and incubated at 27^{o}C, 5 rpm for 35 days.

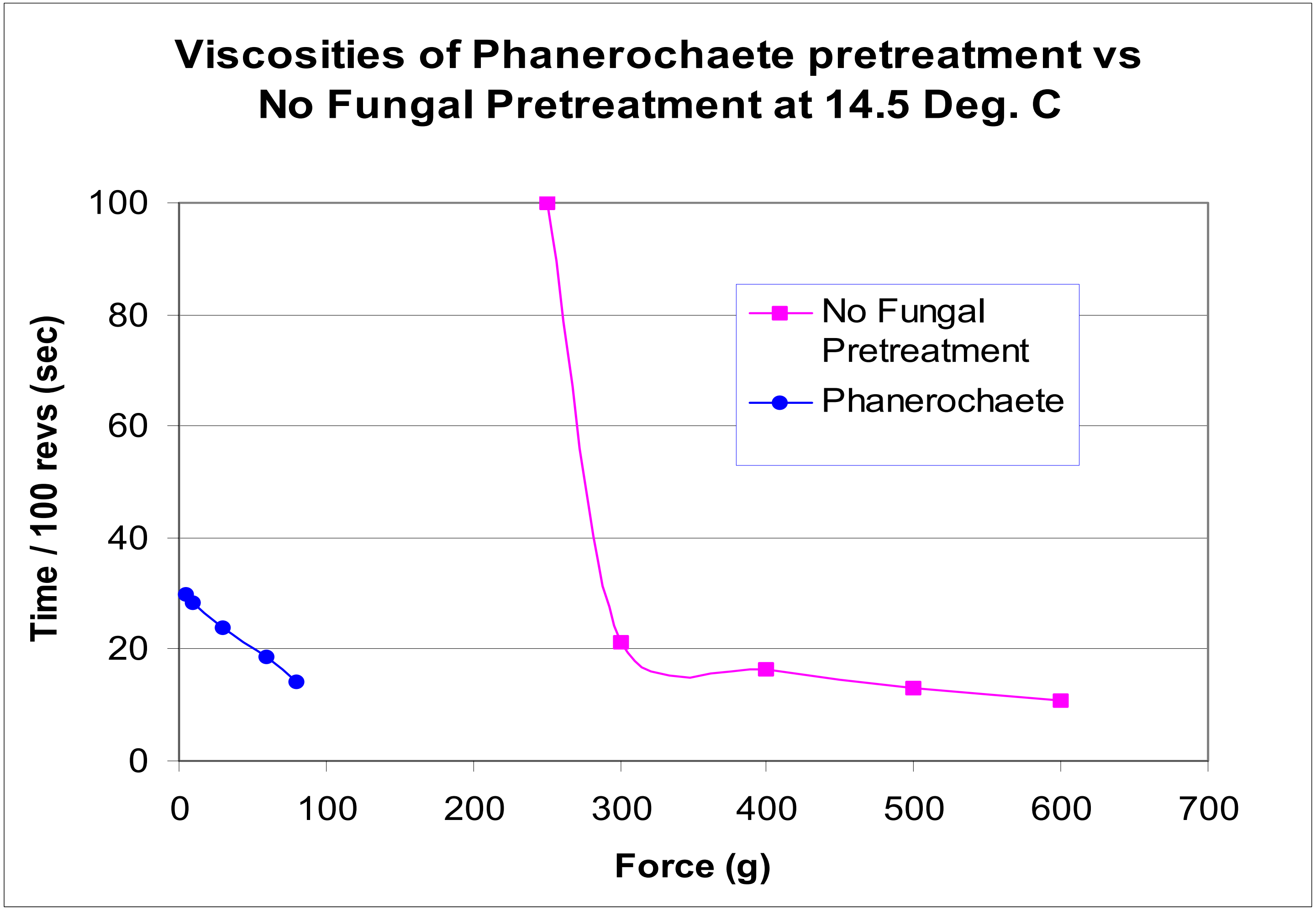
Assays:

- Enzymatic cellulose digestibility of washed shake flask samples
- Viscosity of corn stover slurries after roller bottle pretreatment using the Thomas-Stormer viscometer (Thomas Scientific).

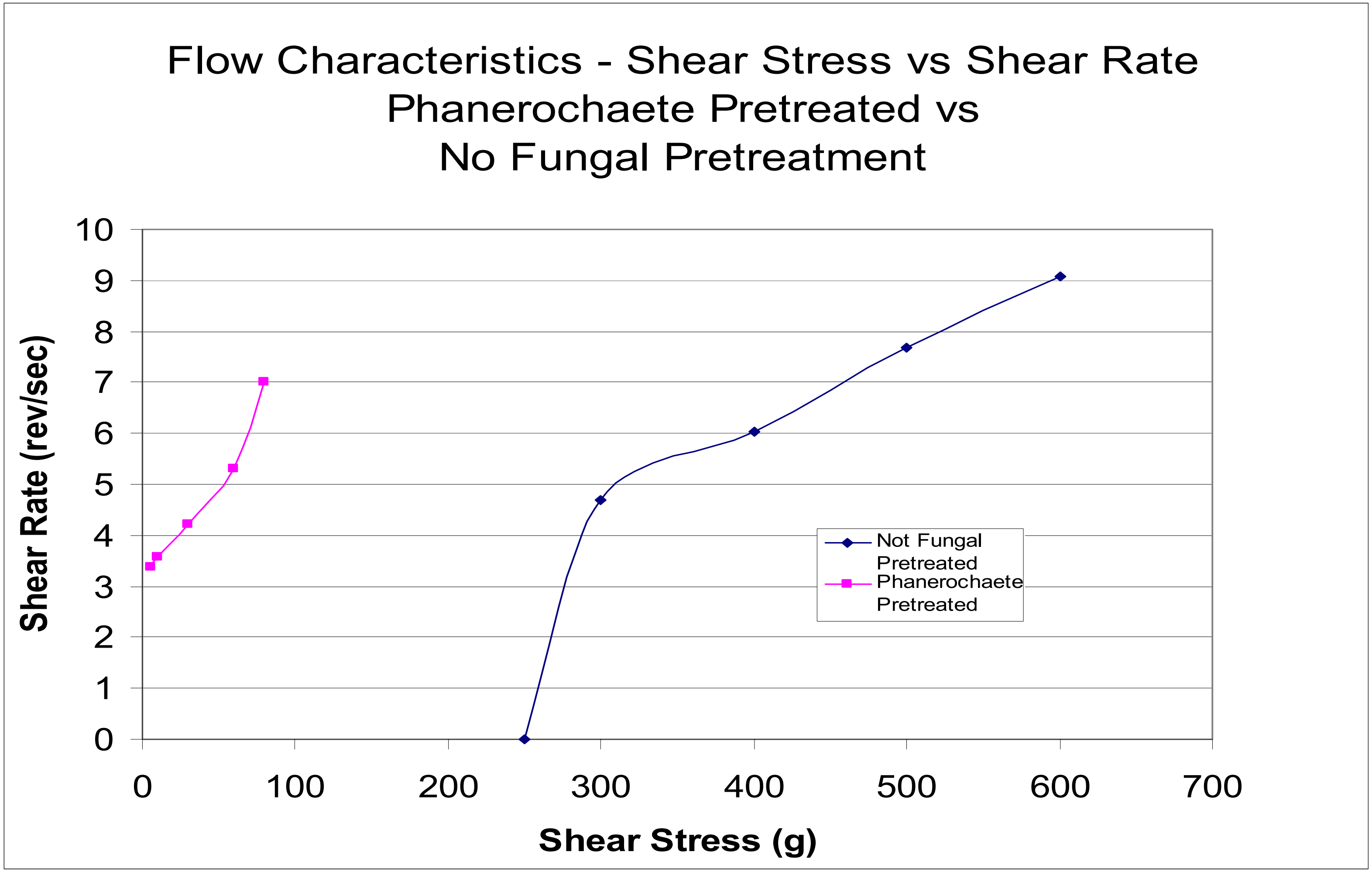
Cellulose Digestibility Results

Flask No.	Enzyme Loading (FPU/g cellulose)	% cellulose digested				Dry Insoluble Solids Loss (weight %)
		16 h	22 h	112 h	136 h	
Digestibility Control: No fungal Culturing and No Incubation						N/A
1	15	0.3	1.2	10.2	9.5	
2	25	0.7	1.4	7.2	9.4	
3	60	8.4	9.6	18.1	16.1	
Incubation Control: No fungal Culture, 29-day Incubation @ 27°C						5.6
4	15	1.2	1.6	3.8	6.9	
5	25	2.2	2.6	5	6.1	
6	60	6.3	6.7	11	12.1	
***Cyathus*-Pretreatment, 29-day Incubation @ 27°C**						8.3
7	15	8.3	10.2	24.6	24.6	
8	25	11.7	13.3	24.4	29.3	
9	60	20.5	22.1	34.0	35.7	
***Phanerochaete*-Pretreatment, 29-day Incubation @ 27°C**						20.0
10	15	0.3	0.5	4.9	3.4	
11	25	2.0	2.3	4.5	5.8	
12	60	5.6	6.1	9.8	12.0	

Viscosity Measurements



Flow Characteristics



Conclusion

- Pretreatment with *Phanerochaete chrysosporium* resulted in:
  - significant reduction of viscosity of stover slurry
  - no apparent change in cellulose digestibility
  - high dry matter loss (20% vs. 5.6% for control).

- Pretreatment with *Cyathus stercoreus* resulted in:
  - 3-4 folds increase in cellulose digestibility
  - moderate dry matter loss (8.3% vs. 5.6% for control).

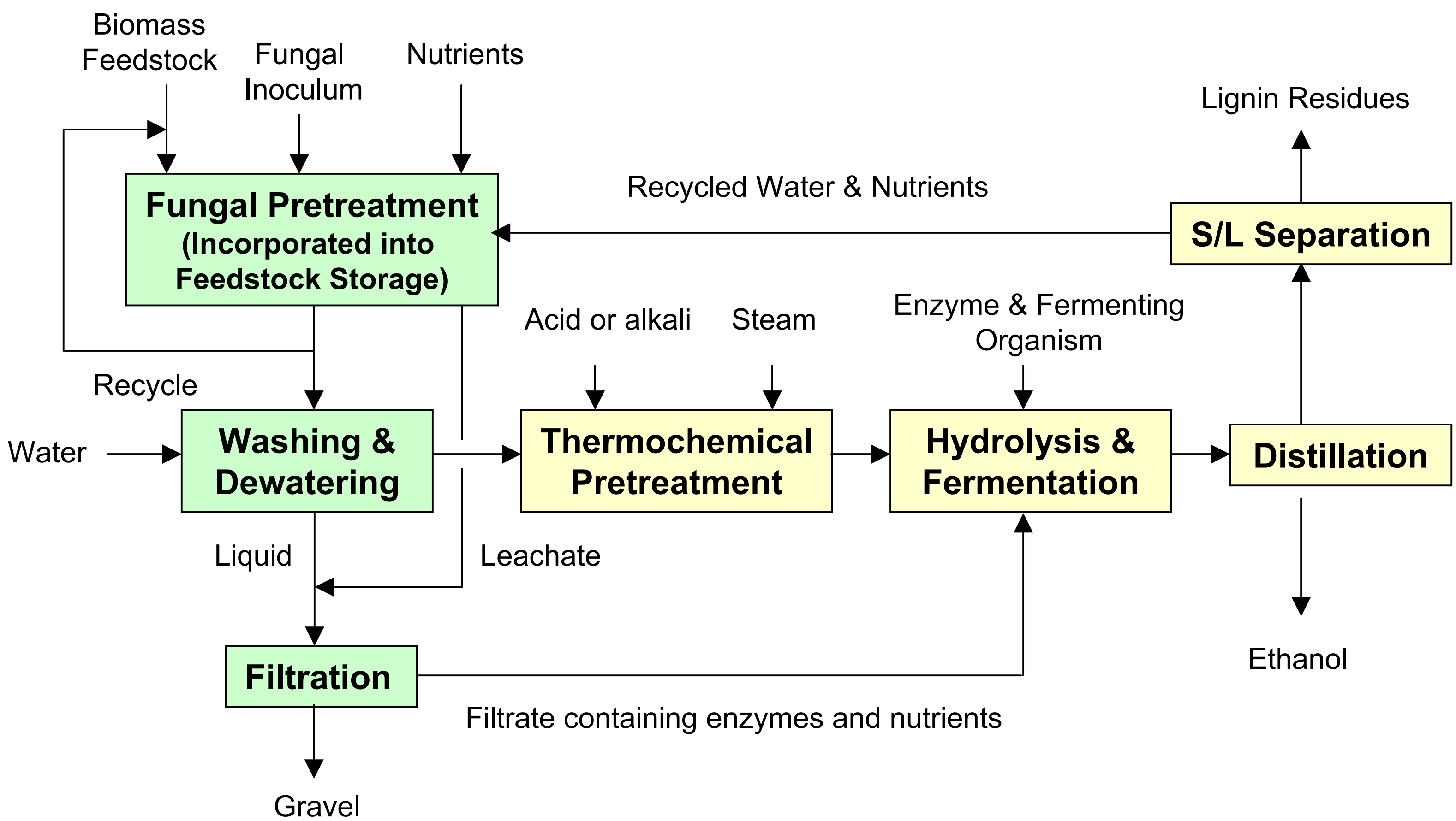
Proposed Performance Criteria for Fungal Pretreatment

1. Low mass loss of feedstock, especially carbohydrates
2. Low nutrients and air requirements
3. Relatively short residence time
4. Robust fungal pretreatment, i.e., the fungi must compete with the natural flora
5. Lower thermochemical pretreatment severity (i.e., reduction in one or more of: temperature, acid/alkali conc., and time)
6. Improve enzymatic cellulose digestibility
7. Not inhibitory to fermenting organisms

Conceptual Bioethanol Production using Fungal Pretreatment

- Fungal pretreatment could be integrated into feedstock storage and handling operations because of low operating temperature.
- Co-culture fungal pretreatments are possibilities.
- One time inoculation or small make-up of culture are possible with recycle of fungal-pretreated feedstock.
- Fungal pretreatment could supplement enzymes for saccharification and fermentation steps. Although the enzyme productivity is low, the total enzyme production could be significant because the entire feedstock stream is pretreated.

Conceptual Bioethanol Production using Fungal Pretreatment



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