DEPARTMENT OF FOOD, AGRICULTURAL AND BIOLOGICAL ENGINEERING

Fungal pretreatment of miscanthus for fermentable sugar production: experimental and techno-economic evaluation

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INTRODUCTION

- **Lignocellulosic biomass:** abundant, renewable feedstock for biofuels production[1], but highly recalcitrant.
  - ✓ **Miscanthus:** perennial grass with high biomass yield and low nutrients and water requirements. Can grow on marginal land.
- **Pretreatment**: reduces recalcitrance of lignocellulosic biomass, enhances enzymatic saccharification
- **Traditional pretreatment:** thermo-chemical methods that use harsh conditions (high temperature and pressure), strong chemicals, and large amounts of water[2].
- **Fungal pretreatment:** alternative process that uses white rot fungi to enhance enzymatic digestibility of lignocellulosic feedstocks[3].
  - ✓ Fungal pretreatment generally requires prior sterilization of the feedstocks to eliminate indigenous microorganisms.

Pros:

Performed in solid-state (no wastewater, no mixing)
Near room temperature and atmospheric pressure
No added chemicals
No inhibitors: no washing/detoxification

Cons:

Low yields
Long residence times
Sensitive to contamination

Needs sterilization?
Low cost?

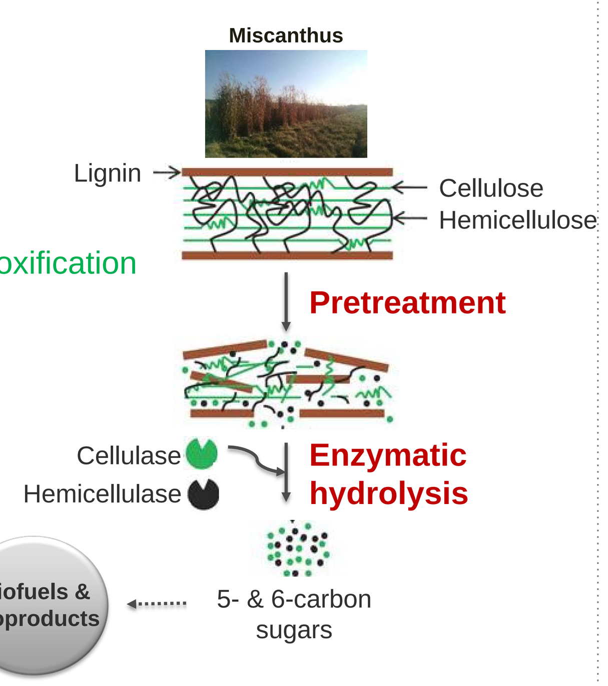


Fig. 1 Miscanthus conversion to biofuels and bioproducts (simplified)

AIM

Investigate the performance and cost-effectiveness of fungal pretreatment of miscanthus, a model lignocellulosic feedstock, for the production of fermentable sugars in a biorefinery context.

METHODS

- **Feedstock:** *Miscanthus* × *giganteus* from Zanesville, OH. Dried at 40ºC and milled.
- **Strain:** *Ceriporiopsis subvermispora* ATCC 96608.
- **Fungal pretreatment experiments:** 1 L reactors. Sterile pretreatment inoculated with pure fungal culture grown in 2% malt extract (**positive control**). Non-sterile pretreatment inoculated with finished material of previous generation (50% w/w). **Negative control:** Unsterilized miscanthus incubated along treatments. Treatments performed in triplicate.
- **Characterization methods:** Compositional analysis and enzymatic digestibility according to NREL protocols[4,5].
- **Data analysis:** Statistical significance evaluated by one way ANOVA (α=0.05), and mean comparisons by Tukey-Kramer test. Software JMP®.
- **Techno-economic analysis:** Software SuperPro Designer® v.9.5.

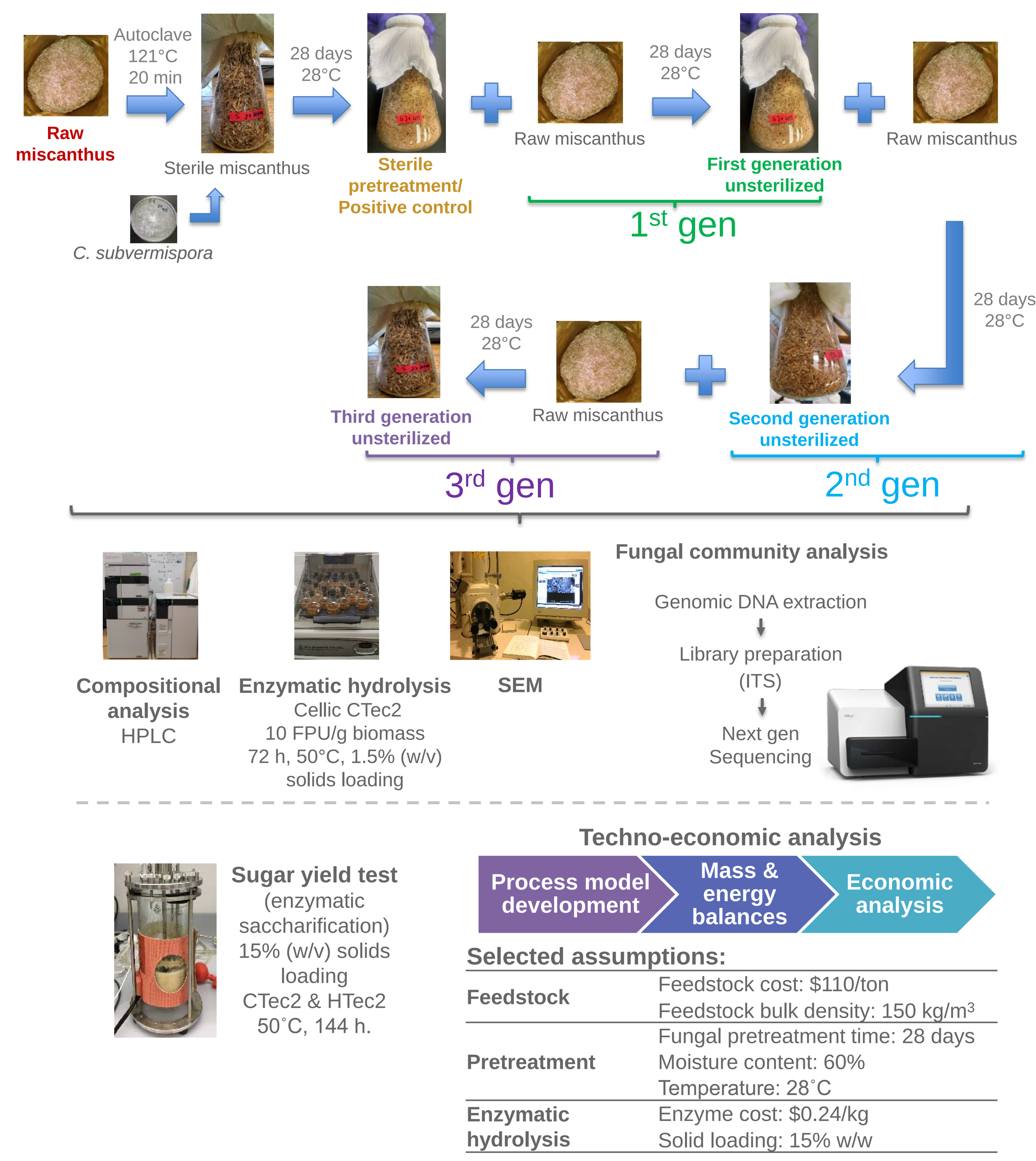
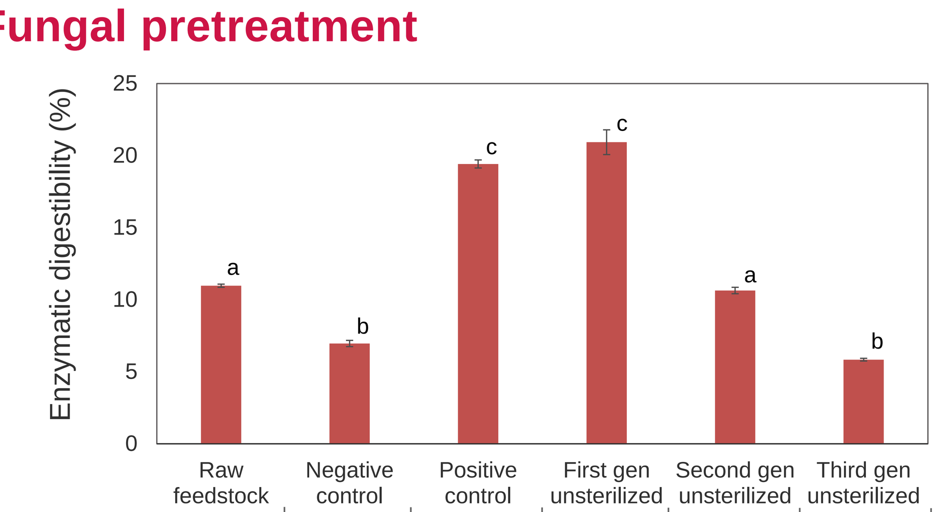


Fig. 2 Methods for sequential fungal pretreatment of miscanthus, enzymatic saccharification and techno-economic analysis

RESULTS AND DISCUSSION

Fungal pretreatment



	Raw feedstock	Negative control	Positive control	First gen unsterilized	Second gen unsterilized	Third gen unsterilized
Lignin degradation (%)	0.5a	5.8b	6.8b	4.2b	0.5a	
Cellulose degradation (%)	6.2a	3.0b	5.1a	7.1a,c	10.7c	
Hemicellulose degradation (%)	4.6a	11.4b	4.1a	7.9c	7.9c	

Note: means marked with same letter did not significantly differ

Fig. 3 Enzymatic digestibility and component degradation after fungal pretreatment of miscanthus

- No difference between the enzymatic digestibility of sterile (positive control) and first generation unsterilized pretreatment.
- Second and third generation pretreatments did not improve enzymatic digestibility.
- Low holocellulose degradation: *C. subvermispora* lacks a strong cellulolytic system[6].

Effects of fungal pretreatment on miscanthus

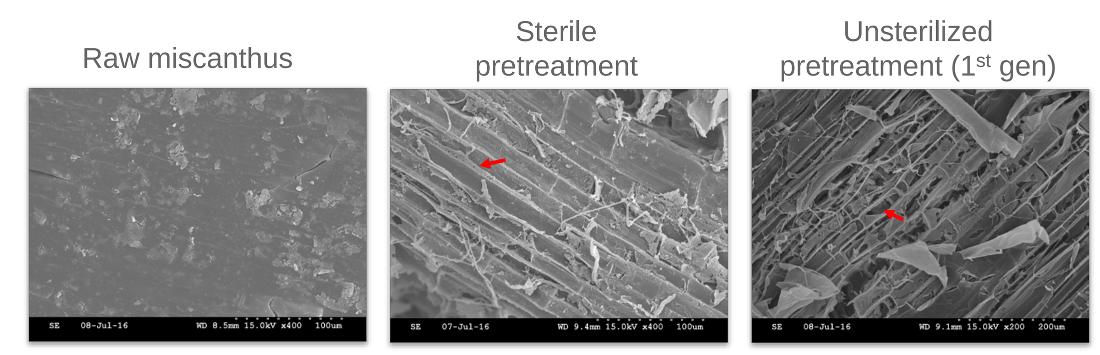


Fig. 4 SEM images of raw and fungal pretreated miscanthus

- Evident increase in porosity and cell wall disruption in accordance with previous research[7].
- More extensive cell wall degradation in the unsterilized pretreatment.

Fungal community composition

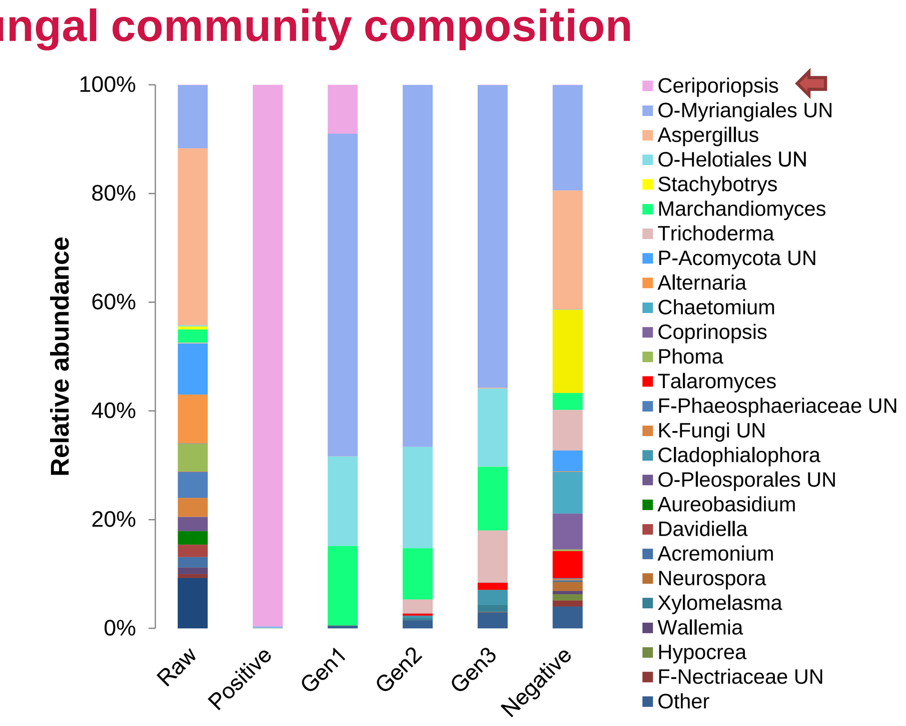


Fig.5 Fungal community - relative abundance at the genus level. UN: unidentified

- *Ceriporiopsis subvermispora* relative abundance decreased from over 99% in the sterilized pretreatment (positive control) to 11% in the first unsterilized generation.
- *C. subvermispora* was out-colonized by other fungi in unsterilized pretreatments.
- Feedstock sterilization is necessary for fungal pretreatment of miscanthus.

Sugar yield

Table 1 Sugar yield after enzymatic saccharification of pretreated miscanthus

Pretreatment	Sugar yield (%)		
	Glucose yield	Xylose yield	Total sugars yield
Fungal – sterilized (positive control)	76.3	40.9	66.2
Liquid hot water	94.4	59.3	84.4
Alkaline	83.8	68.9	79.5

Sugar yield: g of sugar solubilized by enzymatic saccharification/ g of sugar in raw miscanthus

- Fungal pretreatment of miscanthus produced sugar yields comparable to those reported before for pretreatment with *C. subvermispora*[8,9].
- Sugar yield obtained after fungal pretreatment was lower than that of traditional pretreatments.

Techno-economic analysis

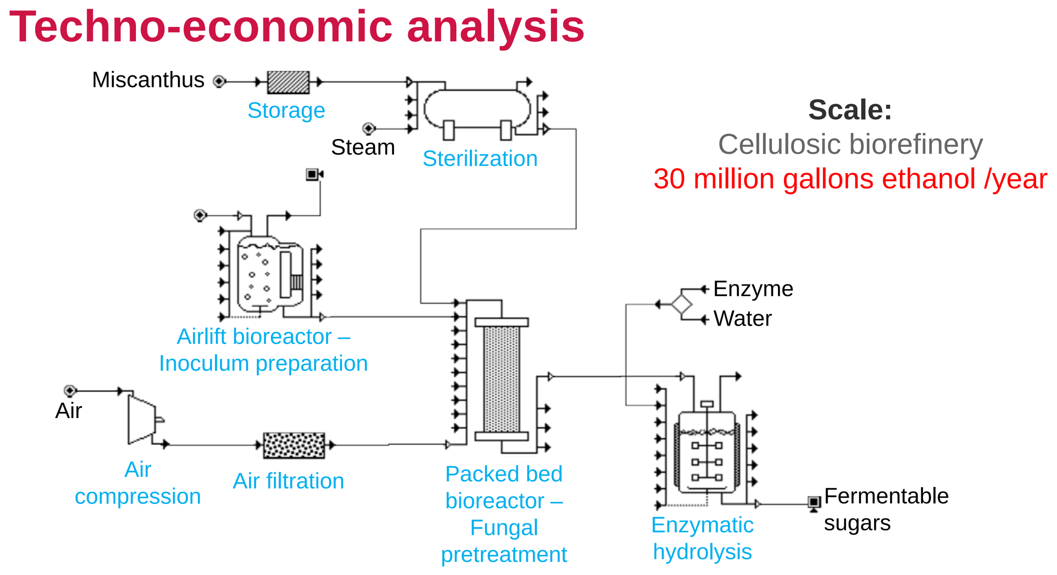


Fig. 6 Overview of the fungal pretreatment process

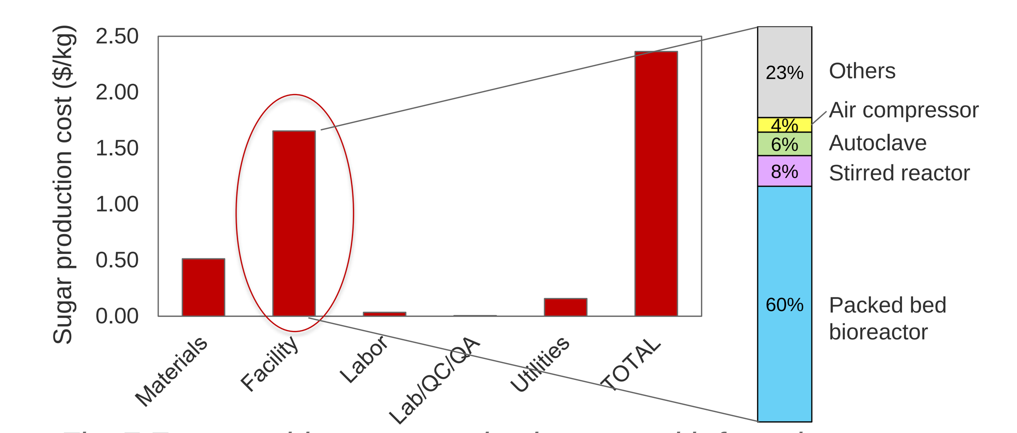


Fig. 7 Fermentable sugar production cost with fungal pretreatment at biorefinery scale

- 70% of the sugar production cost was facility-related, due to the long pretreatment time, low feedstock bulk density, and low yield, that increase need of bioreactor capacity.
- Sugar cost was ~10x that of traditional pretreatments ($0.26/kg)[10].

CONCLUSIONS

- Fungal pretreatment with C. *subvermispora* enhanced the enzymatic digestibility and sugar yield of miscanthus.
- Fungal pretreatment of first generation unsterilized miscanthus (using fungal colonized miscanthus as inoculum) yielded similar results than pretreatment of sterile miscanthus.
- Sequential fungal pretreatment of unsterilized miscanthus (using pretreated miscanthus from previous generation as inoculum) was not feasible: sterilization is necessary.
- Fungal pretreatment of miscanthus is cost-prohibitive at the current state of the technology.
- Future work should focus on increasing the sugar yield and reducing the fungal pretreatment time.

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