INTRODUCTION

- Lignocellulosic biomass: abundant, renewable feedstock for biofuels production1, 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 water2.
- Fungal pretreatment: alternative process that uses white rot fungi to enhance enzymatic digestibility of lignocellulosic feedstocks3.
- 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?

Aim

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

METHODS

- Feedstock: Miscanthus × giganteus from Zanesville, OH. Dried at 40°C 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).
- Pretreatment sterilization: Autoclave sterilization (same time and pressure).
- Fungal pretreatment temperature: 28°C.
- Fungal pretreatment time: 28 days.
- Fungal pretreatment inoculated with pure fungal culture grown in 15% (w/v) solids, 72 h, 50°C, 1.5% (w/v) O2.

RESULTS AND DISCUSSION

Fungal pretreatment

- 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 system4.

Sugar yield

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Glucose yield</th>
<th>Xylose yield</th>
<th>Total sugars yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile</td>
<td>76.3</td>
<td>40.9</td>
<td>66.2</td>
</tr>
<tr>
<td>Sterile</td>
<td>94.4</td>
<td>69.3</td>
<td>84.4</td>
</tr>
<tr>
<td>Sterile</td>
<td>83.8</td>
<td>69.9</td>
<td>79.5</td>
</tr>
</tbody>
</table>

Fungal community analysis

- Ceriporiopsis subvermispora relative abundance decreased from over 99% in the sterilized pretreatment (positive control) to 1% 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.

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

Juliana Vasco-Correa\textsuperscript{a}, Rachel Capouya\textsuperscript{b}, Thomas Mitchell\textsuperscript{b}, Yebo Li\textsuperscript{c}, Ajay Shah\textsuperscript{a}\textsuperscript{*}

Department of Food, Agricultural and Biological Engineering, The Ohio State University, Columbus, OH, USA

Department of Plant Pathology, The Ohio State University, Columbus, OH, USA

QUASAR Energy Group, LLC, Columbus, OH, USA

*Corresponding author: shah.971@osu.edu

BIBLIOGRAPHY