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Green Pretreatment Strategies for Enhanced Microbial Lipid Fermentation and Synergistic High-Quality Lignin Recovery for Next-Generation Integrated Biorefineries

Background/Objective

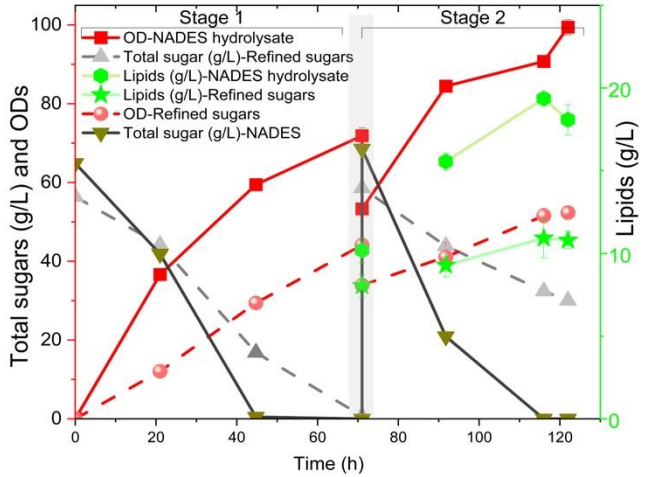
Miscanthus × *giganteus* (Mxg) is a perennial grass being developed as a biomass feedstock for transportation fuels and other chemicals. Current pretreatment methods are expensive and generate microbial inhibitors. To economically address these obstacles, three process strategies were compared for converting Mxg into single-cell oil and extracted lignin intermediates: Hydrothermal (HT) processing and two natural deep eutectic solvents (NADES) pretreatments, ChCl:LA (choline chloride:lactic acid) and ChCl:Gly (choline chloride:glycerol).

Approach

Pretreatments were applied at 10% and 50% solids loading at pre-optimized conditions (NADES: 140°C, 2 h; HT: 190°C, 10 min). Enzymatic hydrolysates, generated at 10% solids using washed and unwashed biomass, were evaluated for microbial lipid production using *Rhodotorula toruloides*. Lignin structure was characterized using 2D-HSQC and ^{31}P NMR.

Results

Overall, NADES pretreatment both enhanced microbial lipid yields and recovered high-quality lignin. Maximum glucose conversions from washed biomass were 84% (ChCl:LA), 53% (ChCl:Gly), and 74% (HT). NADES-derived hydrolysates effectively replaced refined sugars for cultivating *R. toruloides,* achieving ~51% higher biomass and 19 g/L lipid titers within 45 h. ChCl:LA pretreatment enabled high-purity lignin recovery (>89%). Structural analyses showed syringyl (S, 78%), guaiacyl (G, 15%), and p-hydroxyphenyl (p-HPh, 6%) units, with higher p-HPh content and a lower S/G ratio than HT lignin, favoring downstream lignin depolymerization.



Microbial lipid production on enzymatic hydrolysate using *R. toruloides*.

Significance/Impacts

This study advances the economic feasibility of sustainable aviation fuel production from bioenergy crops.

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