Bioabatement to remove microbial inhibitors from *Miscanthus giganteus* hydrolysates for enhanced butanol fermentation

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**Abstract**

The recalcitrant nature of cheap lignocellulose warrants pretreatment process to disrupt the lignin matrix and expose the carbohydrate fraction to enzymatic saccharification. Generation of lignocellulose-derived microbial inhibitory compounds (LDMICs) during the pretreatment process undermines large-scale utilization of biomass for biofuel (e.g., butanol) production. LDMICs are derived from lignin (e.g., vanillin), cellulose (e.g., 5-hydroxy-methylfurfural [HMF]), and hemicellulose (e.g., acetic acid) fractions of lignocellulose. These compounds impair butanol fermentation by disrupting the growth of butanol-producing *Clostridium beijerinckii* through diverse mechanisms including perturbation of redox and energy state of the cell, inhibition of glycolytic enzymes, and damage to cell membrane, nucleic acids and organelles. Although LDMICs can be removed from lignocellulosic biomass hydrolysates (LBH) by physicochemical methods, these methods increase the overall butanol production cost. Bioabatement, a cost-effective alternative, employs microorganisms that selectively metabolize LDMICs in the presence of fermentable sugars. In this study, we demonstrate the ability of the bacterium *Cupriavidus basilensis* ATCC®BAA-699 to metabolize pure LDMICs and *Miscanthus giganteus* biomass hydrolysate (MH)-associated LDMICs. Notably, MH was generated by dilute-acid (2% H₂SO₄) pretreatment at 15% biomass solids loading in a reactor at 180°C and 150 psi for 1 h. The hydrolysate was then detoxified by *C. basilensis* prior to enzymatic hydrolysis to release fermentable sugars. Acetone-butanol-ethanol (ABE) fermentation of *C. basilensis*-detoxified MH resulted in ~70% inhibition in ABE production in control, suggesting disruption of transition from acidogenesis to solventogenesis.

**Introduction**

**Materials and methods**

**Results**

**Specific aims**

- To detoxify *Miscanthus giganteus* lignocellulosic biomass hydrolysates (MH) using *Cupriavidus basilensis* ATCC®BAA-699.
- To evaluate the fermentability of detoxified and non-detoxified MH hydrolysates.

**Conclusions and Discussion**

- We demonstrate for the first time, the use of *C. basilensis* ATCC® BAA-699 to remove LDMICs from LBH and enhance butanol fermentation.
- These results substantiate the hypothesis that *C. basilensis* ATCC® BAA-699, which grows on pure LDMICs as sole carbon substrate(s), can be used to detoxify biomass hydrolysates for enhanced butanol fermentation.
- Loss of fermentable sugars due to co-utilization of LDMICs and sugars by this bacterium was minimized by adopting a bioabatement design wherein pretreated biomass was first detoxified (~12 h) prior to enzymatic hydrolysis.
- Pretreatment conditions in which less glucose is generated will further reduce loss of sugars to *C. basilensis* during biological abatement of LDMICs.

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**References**


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