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Tolerance of Engineered Rhodosporidium toruloides to Sorghum Hydrolysates during Batch and Fed-Batch Lipid Production

Background/Objective

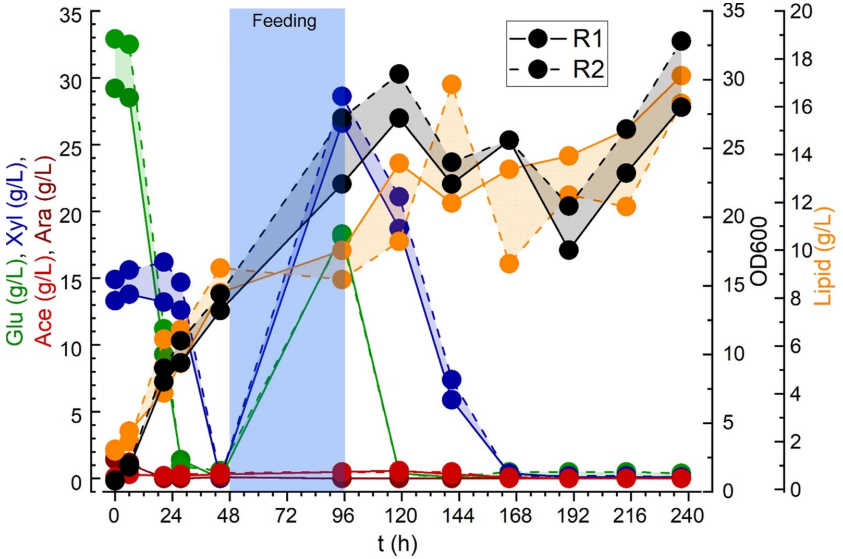
Oleaginous yeasts are promising candidates for bioconversion of lignocellulosic feedstocks, but their growth on these substrates can be inhibited by upstream pretreatment and enzymatic hydrolysis conditions. Previous studies indicate a high citrate buffer concentration during hydrolysis inhibits cell growth and ethanol yield in *Saccharomyces cerevisiae*. In this study, an engineered *Rhodosporidium toruloides* strain with enhanced lipid accumulation was grown on bioenergy sorghum hydrolysate prepared using high and low citrate buffer concentrations.

Approach

A previously engineered *R. toruloides* strain with enhanced triacylglycerol (TAG) accumulation was grown in bioreactors on sorghum hydrolysate prepared via hydrothermal pretreatment and enzymatic hydrolysis at high and low citrate buffer concentrations. Wash water prepared from enzymatic hydrolysis residue was tested as a seed culture medium, and lipid production was evaluated in both batch and fed-batch modes in lab-scale bioreactors.

Results

Both hydrolysis conditions resulted in similar sugar recovery rates and concentrations. No significant differences in cell growth, sugar utilization rates, or lipid production rates were observed between the two citrate buffer conditions during batch fermentation of *R. toruloides*. Under fed-batch growth on low-citrate hydrolysate, a lipid titer of 16.7 g/L was obtained.



Growth curve for *R. toruloides* cells grown by fed-batch conditions with diluted sorghum hydrolysate and 0.5 mM citrate buffer media.

Significance/Impacts

As this process is scaled up, it has the potential to reduce materials cost in the upstream bioprocessing without adversely affecting downstream product yields. Key challenges identified include slow xylose utilization in *R. toruloides* and residual nitrogen causing reduced lipid titers, and these may be addressed with additional strain engineering.

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