Effects of Furfural and HMF on the Co-fermentation of Glucose and Xylose from Pretreated Lignocellulosic Biomass by Recombinant Yeast

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Introduction

Pretreatment of lignocellulosic biomass, while improving enzymatic digestibility, can also produce fermentation inhibitors such as furfural and HMF. Both furfural and HMF can decrease the fermentability and the ethanol yields from sugars derived from lignocellulose. This paper reports a systematic study of the effect of furfural and HMF on the fermentation of both glucose and xylose to ethanol by the recombinant yeast S. cerevisiae 424A(LNH-ST). Fermentations were run with furfural, HMF, or both in a control solution of YEP with glucose and xylose as co-substrates. Inhibitor concentrations were varied and ranged from 0 to 40 g/L. Further experiments varied inhibitor concentration in the presence of a single substrate, either glucose or xylose. Batch fermentations were carried out for 48 hours in 300 ml sidearm flasks at 30°C and 200 rpm with periodic sampling for analysis by HPLC. Our results show that concentrations of either furfural or HMF below about 5 g/L cause negligible inhibition for yeast cells in early stationary phase. We confirm that furfural is more inhibitory than HMF. Lastly, xylose fermentation is more sensitive to these inhibitors than glucose for fermentation to ethanol.

Materials and Methods

• Pretreatment was performed in 300 ml baffled Erlenmeyer flasks at 30°C and 200 rpm in a shaking water bath. The yeast were grown in 100 ml YEPD until reaching 500-550 KU and then glucose, xylose, furfural and/or HMF were added. The flasks were then sealed with butyl stoppers to allow fermentation to be carried out under largely anaerobic conditions.

• Samples were analyzed by HPLC using a Waters 2487 Refractive Index Detector, Waters 2414 Absorbance Detector, and an Agilent 3395 or Hewlett Packard HP3396G Integrator. The mobile phase was degassed 5 mM H2SO4 filtered through 0.2 nylon filter (Millipore).

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Results and Conclusion

• Fermentation more sensitive to furfural than HMF  
• Maximum tolerant concentrations lower for furfural than HMF  
• Xylose utilization is more strongly affected than glucose utilization  
• Maximum tolerant concentration lower for xylose than glucose  
• Large toxic power constant (5.1 – 5.3) for furfural indicates utilization and modeling using Levengpiel Toxic Function  
• An increase from 2 – 4 g/L (0.22 g/g – 0.44 g/g) results in approximately a 15% decrease in glucose and a 22% reduction in xylose utilization rates

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Modeling

\[
\frac{dx}{dt} = \frac{V_{max}}{K_{x}} \left( \frac{G}{K_{G} + G} \right) - x \frac{K_{x}}{K_{x} + G} \\
\frac{dy}{dt} = \frac{V_{max}}{K_{y}} \left( \frac{F}{K_{F} + F} \right) - y \frac{K_{y}}{K_{y} + F} \\
\frac{dc}{dt} = \frac{V_{max}}{K_{c}} \left( \frac{G}{K_{G} + G} \right) - c \frac{K_{c}}{K_{c} + G} \\
\frac{dG}{dt} = -x v_{G} - y v_{F} - n_{x} G - n_{y} F - n_{c} c \\
\frac{dF}{dt} = -x v_{G} - y v_{F} - n_{x} G - n_{y} F - n_{c} c \\
\frac{dH}{dt} = -x v_{G} - y v_{F} - n_{x} G - n_{y} F - n_{c} c \\
\frac{dH}{dt} = -x v_{G} - y v_{F} - n_{x} G - n_{y} F - n_{c} c
\]

\[\begin{align*}
T & = \frac{1}{K_{x}} \\
F_{max} & = \frac{T}{K_{x}} \\
G_{max} & = \frac{T}{K_{y}} \\
x_{max} & = \frac{T}{K_{c}}
\end{align*}\]