

## Increased production of isobutanol from xylose through metabolic engineering of *Saccharomyces cerevisiae* overexpressing transcription factor Znf1 and exogenous genes

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### Abstract

To improve isobutanol production from xylose, *S. cerevisiae* was engineered to overexpress Znf1, xylose reductase (XR), xylitol dehydrogenase (XDH), xylulokinase, and isobutanol-pathway enzymes. Using a 5 L bioreactor with added  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , the engineered strain produced  $14.81 \pm 0.40$  g/L isobutanol from 10% xylose, corresponding to 155.88 mg/g consumed and a 264.75% yield improvement. Proteomic and transcriptomic analyses confirmed pathway activation and increased tolerance to lignocellulosic inhibitors.



**Winpact Model: FS-05**

### Introduction

Biorefinery utilization of xylose remains challenging in *S. cerevisiae*. The transcription factor Znf1 has been shown to enhance non-fermentable carbon source metabolism. Coupling Znf1 overexpression with expression of xylose-pathway and isobutanol-production genes could improve yields. This study aimed to engineer *S. cerevisiae* and optimize fermentation conditions for scalable isobutanol production in a 5 L bioreactor.

### Materials and Methods

Biorefinery utilization of xylose remains challenging in *S. cerevisiae*. The transcription factor Znf1 has been shown to enhance non-fermentable carbon source metabolism. Coupling Znf1 overexpression with expression of xylose-pathway and isobutanol-production genes could improve yields. This study aimed to engineer *S. cerevisiae* and optimize fermentation conditions for scalable isobutanol production in a 5 L bioreactor.

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## Results

- Isobutanol titer reached  $14.81 \pm 0.40$  g/L (155.88 mg/g xylose) with FeSO<sub>4</sub> supplementation.
- Yield improved by ~264.75% compared to control.
- Proteomics indicated activation of valine biosynthesis, Ehrlich pathway, iron–sulfur cluster pathways.
- Transcriptomics confirmed Znf1-mediated upregulation of key genes in xylose utilization and stress tolerance.

## References

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