Isobutanol production in *Escherichia coli*

Metabolic engineering for biofuel production from lignocellulosic biomass

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INTRODUCTION

One of the biggest challenges of the current society is developing energy sources that allow for both reducing dependence upon fossil fuels and helping to satisfy the growing energetic demand. Among these alternative energy sources biofuels are one of the most developed, with advanced biofuels such as isobutanol and production processes from non-edible biomass, such as lignocellulosics, becoming more relevant.

AIM: Review metabolic engineering strategies in *Escherichia coli* to optimize isobutanol production and adapt it to the usage of lignocellulosic biomass as a substrate.

	Ethanol	Isobutanol	Gasoline
Energy density (MJ/L)	21	29	32
Avg octane number	116	110	90
Hygroscopicity	High	Low	Low
Corrosivity	High	Low	Low
Fits current infrastructure?	No	Yes	Yes

Isobutanol is an advanced biofuel, showing better properties than ethanol (the dominating biofuel currently) and presenting itself as a promising alternative to fossil fuels.

Saccharomyces cerevisiae	*Escherichia coli*
Low isobutanol titer	High isobutanol titer
High expression protein level	Very high expression protein level
Homologous isobutanol pathway	Non homologous isobutanol pathway
Not able to metabolize 5C sugars	Able to metabolize 5C sugars
High tolerance of high isobutanol concentrations	Low tolerance of high isobutanol concentrations

S. cerevisiae is the most used microorganism to produce alcohols, but *E. coli* offers advantages in terms of isobutanol titer and processing of lignocellulosic biomass (5C sugars). However, *E. coli* has to be engineerized in order to **have an isobutanol synthesis pathway** and to **improve its tolerance to high isobutanol concentrations**.

Improving isobutanol production in *E. coli*

Ehrlich pathway:

glucose

Glycolysis pathway → 2 NADH

pyruvate

↓ ALS

2-acetolactate

2 NADP+ ↓ KAR

2,3-dihydroxy-isovalerate

↓ DHAD

2-ketoisovalerate

↓ KDC

isobutyraldehyde

2 NADP+ ↓ ADH

isobutanol

Modifications of the pathway:

- ALS → AlsS (*Bacilus subtilis*) expression
- KAR → Overexpression; Mutagenesis to make it NADH-dependent
- DHAD → Overexpression
- KDC → KivD (*Lactococcus lactis*) expression
- ADH → AdhA (*L. lactis*) expression; AdhA mutagenesis to improve its catalytic efficiency

Gene disruption

Disruption of genes competing for pyruvate and causing the synthesis of secondary products alternative to isobutanol:

pflB, *ldhA*, *pta*, *adhE*, *frdAB*, *frn*

Improving isobutanol tolerance in *E. coli*

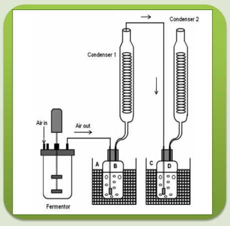
GENETIC ENGINEERING

Targeting genes involved mainly in:

- Post-transcriptional regulation processes
- High centrality nodes of biochemical networks

REMOVING ISOBUTANOL FROM THE MEDIUM

through **gas stripping**



Improving adaptation to lignocellulosics in *E. coli*

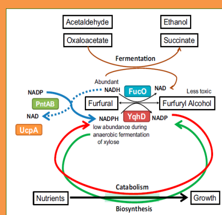
1) Improving furfural tolerance:

- *yqhD* disruption: NADPH consumption avoided
- *fucO* overexpression: reduction of furfural components without NADPH consumption

2) Improving of lignocellulosic degradation-derived sugars:

- Pentose degradation: *pstG* disruption avoids CCR
- Cellobiose degradation : expression of extracellular β-glicosidase *BglC* from *T. fusca*

1)



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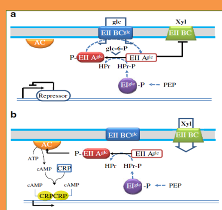

Conclusions

- *E. coli* production strains with high yield and high isobutanol titer are obtained through metabolic engineering.
- **Future:** further adaptation to lignocellulosic biomass needs to be done.
- **Industrial application:** although S. *cerevisiae* is the hegemonic microorganism for industrial alcohol production, *E. coli* proves to be a valid alternative for isobutanol production.

References

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