Metabolic engineering for biofuel production

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Introduction

The use of petrol as a main energetic source has severe problems and controversies. Environmental pollution, both social and economical inequality and fuel depletion carry with them the necessity of new and sustainable alternatives.

In this bibliographic report I explore biotechnology tools to produce biofuel in order to compete with conventional hydrocarburs, especially metabolic and genetic engineering. This technique is able to transform microorganisms (bacteria, yeast and fungi) into cellular factories that provides us large amounts of bioalcohols from cheap raw materials. Important research groups and giant chemical factories, in joint venture with biotech companies, are trying to develop this technology using different processes. This study focusses on the isobutanol synthesis using *Saccharomyces cerevisiae*.

Goal

The goal of this report consist of an overview of the best research and industrial strategies of metabolic engineering on microorganisms to produce biofuels. In the same way, the limitations and future needed improvements of the technology are also studied.

Tools

New computer technologies provide huge knowledge that allow us to improve quickly and qualitatively the conventional engineering by avoiding the bottlenecks. These tools are part of the *omic* sciences: genomics, transcriptomics, proteomics, metabolomics and fluxomics.

Isobutanol

	Ethanol	Isobutanol
Energy density	29,7 MJ/kg	36,1 MJ/kg
Average octane number	116	110
Vapor pressure	High	Low
Higroscopicity	High	Low
Corrosivity	High	Low
Compatible with current infrastructure	Low	High

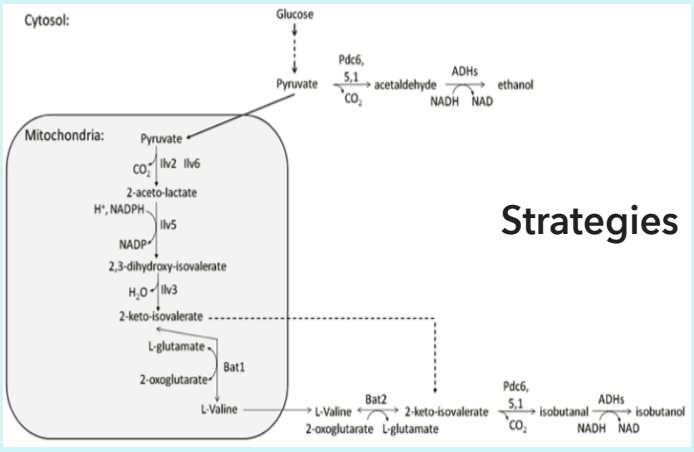
Ethanol is the most up-to-date widely produced biofuel.
However, Isobutanol production is the really promising alternative to petrol.

Saccharomyces cerevisiae

Escherichia coli	*Saccharomyces cerevisiae*
Molecular characterization advanced	Molecular characterization advanced
High alcohol titer	Low alcohol titer
No homologous isobutanol pathway	Homologous isobutanol pathway
Low tolerance in high alcohol concentration	High tolerance in high alcohol concentration
Low robustness in industrial conditions	High robustness in industrial conditions

In spite of being bacteria *Escherichia coli* the principal microorganism in industry, most succesfully biofuel companies use the yeast *Saccharomyces cerevisiae*.

Genetic and metabolic engineering



Strategies

1. Research

Two main ways:

a) Overexpression of gens from valine metabolism

b) Cytosolic re-localization of valine synthesis

Engineering steps	Overexpressed genes	Titer increase from reference
1	$ILV2_{mitochondrial}$, $ILV3_m$ and $ILV5_m$	6-fold
2	$ILV2_m$, $ILV3_m$, $ILV5_m$ and $ILV6_m$	2-fold
3	BAT2	13-fold
4	$ILV2_{cytosolic}$, $ILV3_c$ and $ILV5_c$	13-fold $_{optimized\ codon}$
	KDC and ADH	35-fold $_{non\ optimized\ codon}$

2. Industry

Company	Engineering
Gevo	• Cytosolic re-localization of isobutanol synthesis • Deletion of competitive pathways: glycerol and ethanol
Butalco	• Heterologous enzyme replacements in Ehrlich pathway • Deletion of genes on substrate competition
Butamax	• Cytosolic re-localization of isobutanol synthesis • Deletion of genes on substrate competition • Introduction of heavy promoters in weak expression genes

3. Future

a) Cofactor and amino acid balance

b) Improve the robustness against product inhibition

c) Codon optimization from catabolic to anabolic use

Yeast produces isobutanol through ILV, KDC and ADH genes in the fermentative Ehrlich Pathway

Conclusion

Metabolic and genetic engineering is not only the present of biofuel production but also the future. Overcoming the process bottlenecks and being competitive in fuel production, the energy paradigm change is not far away.

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