

Chair of Bioprocess Engineering

Engineering of synthetic C4 pathway for using ethylene glycol

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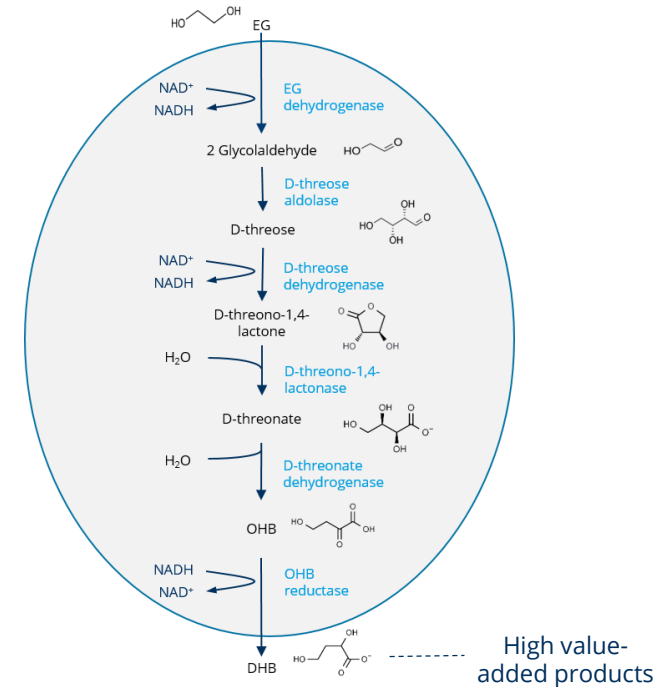
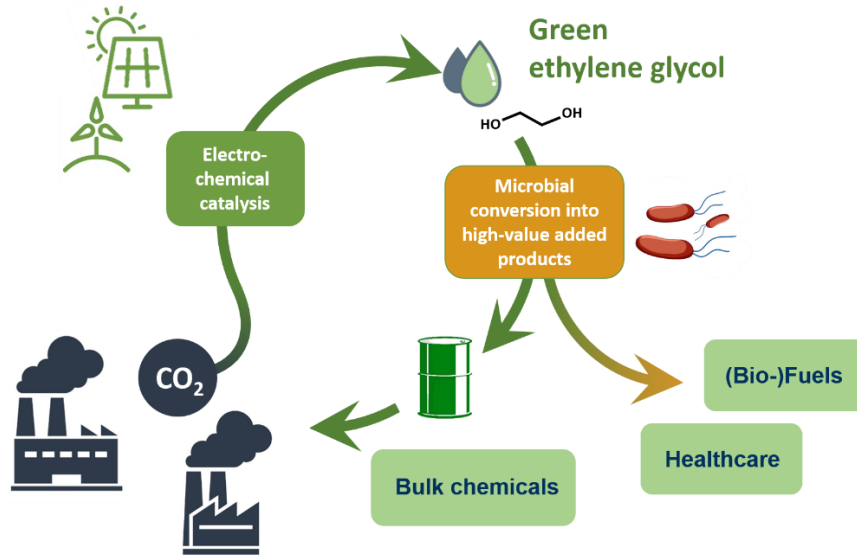
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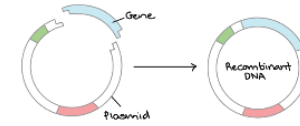
Motivation

Ethylene glycol as promising alternative substrates for microbial conversion



Synthetic pathway for the carbon-conserving conversion of EG into DHB

Applied techniques

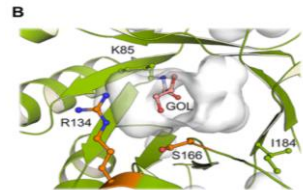


Enzyme engineering

- Saturation mutagenesis
- Rational enzyme design

Enzyme screening

- Plasmid construction
- Protein expression
- Enzymatic assay



(Thorell *et al.*, 2002)



tinyurl.com/2p9eaaw7

Strain engineering

- ^{13}C metabolic flux analysis



Advances in this project

Enzyme level:

- Enzymes for all metabolic steps are available.
- Assimilation of EG into DHB through the assembled metabolic pathway was demonstrated in vivo.
- Two rate-limiting steps with:
 - (D)-threose aldolase
 - (D)-threose dehydrogenase

Microorganism level:

- The objective is to transfer the engineered metabolic pathway into *Pseudomonas . putida*

In-vitro selection and characterization of enzymes



In-vivo validation of the assembled metabolic pathway



Optimization of the enzymatic reactions



Optimization of the productivity concerning the producer strain

Advances in this project

Enzyme engineering

Molecular biology including cloning/plasmid construction, targeted and random mutagenesis, enzymatic assays, development of high throughput enzymatic assays

Strain engineering

Metabolic engineering of *P. putida*

Topics are suitable for Internships, Bachelor/Master/Diploma-projects