Metabolic Engineering and Dynamic Modelling of *Escherichia coli* for the Production of Chemicals from Renewable Resources (MEMORE)

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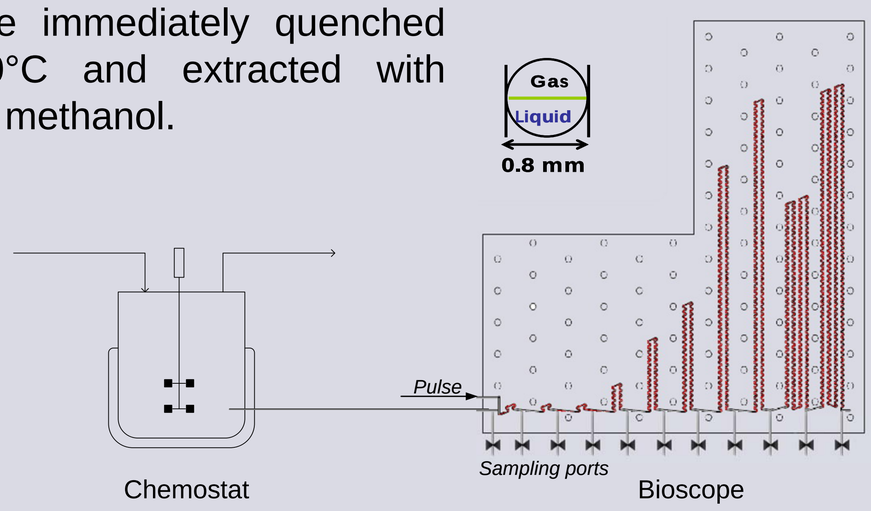
Abstract: The aim of the MEMORE project is to develop methodologies and provide tools and know-how to speed up and improve the development of microbial production strains for productions of useful compounds. This multidisciplinary project involves four laboratories that collaborate to bring together the required expertise of relevant scientific domains in order to develop dynamic metabolic models of central metabolism of micro-organisms. With this approach we aim to replace the more commonly used 'trial and error' approach for optimization of industrial production processes with the more rational metabolic modelling approach.

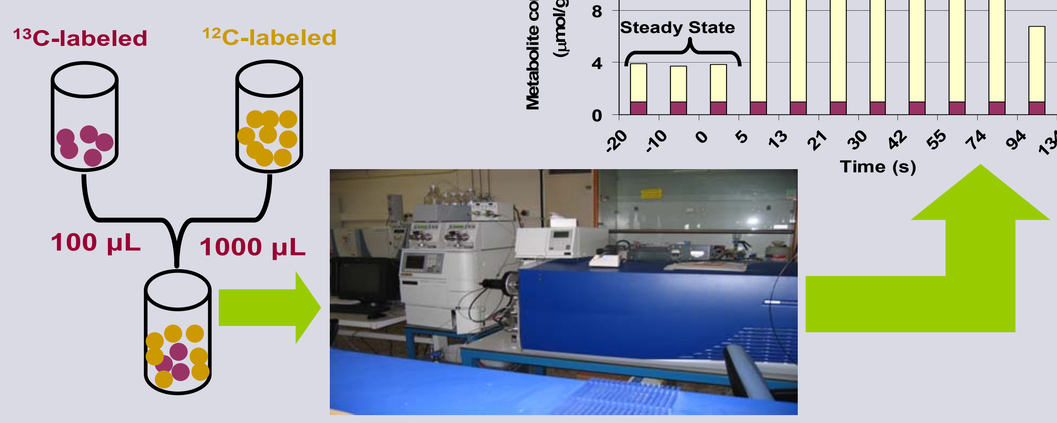
Fermentation technology

By means of a plug flow reactor (Bioscope) broth derived from chemostat cultures are pulsed with different substrates. At different time-intervals samples can be taken. These samples are the immediately quenched at -40°C and extracted with boiling methanol.



Analysis methodology

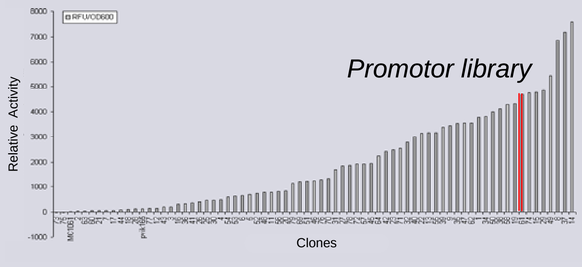
Quantification of intracellular metabolites of central metabolism using Isotope Dilution Mass Spectroscopy (IDMS) and uniformly labeled ^{13}C internal standards [1].

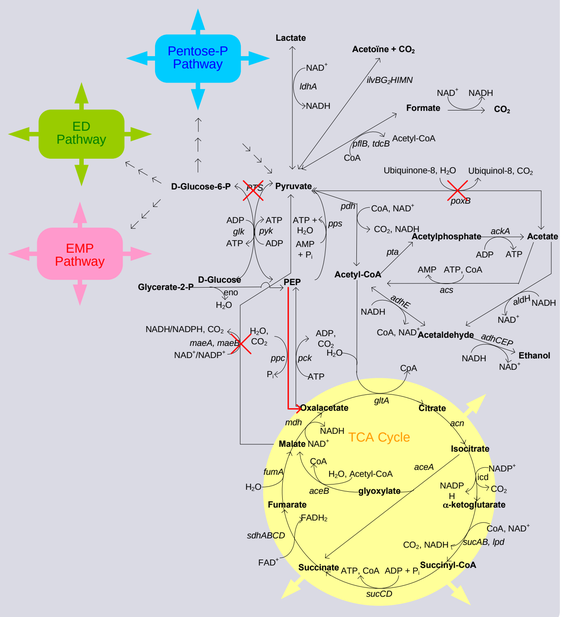


Wu L, Mashego MR, et al., 2005, *Anal. Biochem.*, 336(2):164-71.

Genetic engineering

Using the one step inactivation of chromosomal genes [2] and an extensive promotor library [3], genes can easily be knocked out or overexpressed.
The effect on the metabolism of each modification is then tested experimentally and used for model validation.

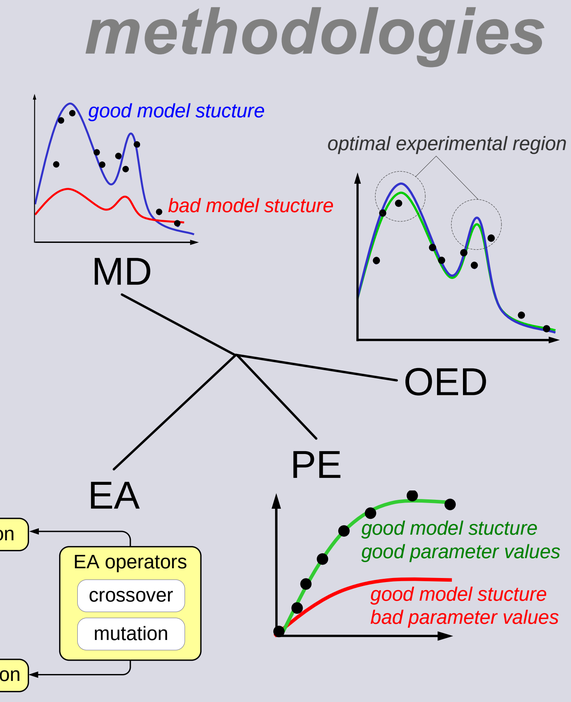




[2] Datsenko KA, Wanner BL, 2000, *PNAS*, 97:6640-6645.
[3] De Mey M., 2007, PhD-thesis, Ghent University.

Mathematical methodologies

A new dynamic metabolic model structure identification and a robust parameter estimation procedure will be developed to come up with kinetic equations of reaction rates, which are valid in vivo. Candidate models are identified using Evolutionary Algorithms (EA). Optimal experimental design is used for model discrimination (OED-MD) and parameter estimation (OED-PE).



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