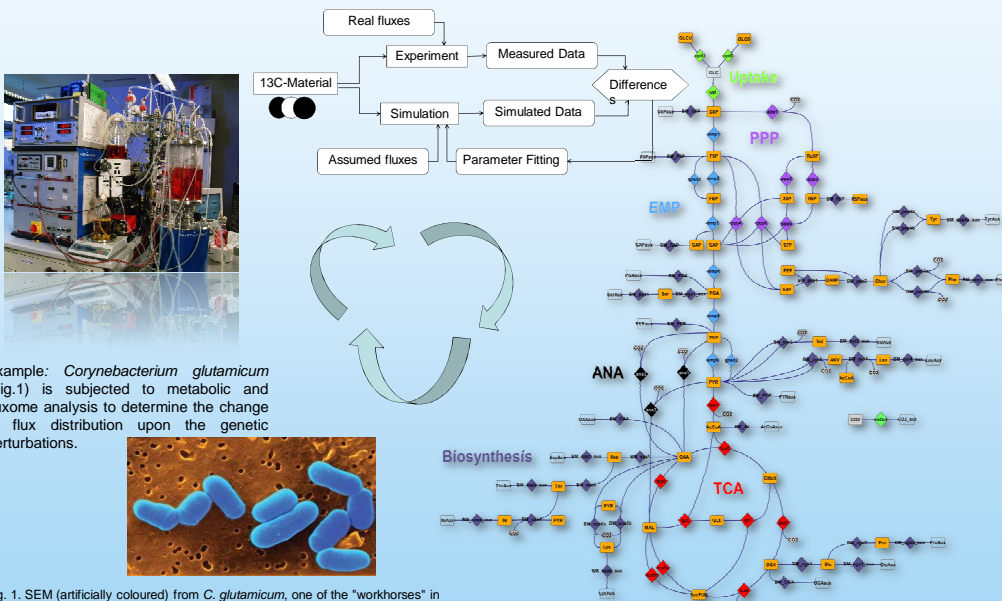


Metabolic flux analysis for strain characterization

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Metabolic flux analysis (MFA) has become a well-established diagnostic tool in metabolic engineering in recent years. It is invaluable for characterizing different strains or different physiological states of a microorganism. The development of MFA started with a purely stoichiometric approach which requires only measured extracellular fluxes (substrate uptake, biomass production, product formation, CO₂ evolution, etc.) as input data. Some major drawbacks of the purely stoichiometric approach led to the development of the ¹³C labelling MFA, a method which works particularly well for complex metabolic networks, especially for resolving problems related to bidirectional or parallel reaction steps. The ¹³C method is based on a carbon-labelling experiment (CLE) where a specifically ¹³C-labeled substrate like [1-¹³C]glucose is used. This labelled material is then distributed over the metabolic pathways until a stationary state of label enrichment is reached in each intracellular metabolite pool. In this stationary state, the label enrichment is measured by using different NMR or MS instruments. ¹³C MFA is much more powerful and reliable than stoichiometric MFA.



Example: *Corynebacterium glutamicum* (Fig.1) is subjected to metabolic and fluxome analysis to determine the change of flux distribution upon the genetic perturbations.

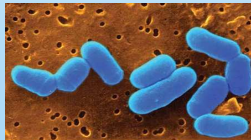


Fig. 1. SEM (artificially coloured) from *C. glutamicum*, one of the "workhorses" in industrial biotechnology. It is used for production of more than 2 million tons of amino acids per year. Courtesy of Michael Bott / Research Center Jülich.

Fig. 2. Metabolic network of *E. coli*

The metabolic flux analysis can be used to directly monitor the benefit of the corresponding genetic modifications. The result is a flux map (Fig. 2) that shows the distribution of anabolic and catabolic fluxes over the metabolic network. Based on such a flux map or a comparison of different flux maps, possible targets for further genetic modifications might be identified, and the result of an already performed genetic manipulation can be quantitatively evaluated together with a deep knowledge of the cellular energy metabolism.

For the metabolic analysis a real-time computer controlled process will be established. An automated and highly integrated system for fast-sampling, quenching and extraction of metabolites of microorganisms recently constructed in our group will be used to study the mutants under physiological dynamic conditions.

For the fluxome analysis, the intracellular and extracellular metabolic changes in small scale fermentations will be evaluated with MS based ¹³C labelling analysis and computer aided design for flux calculation. The flux studies will further include analysis of substrates, amino acids and by-products by HPLC and LC-MS as well as analysis of anabolic fluxes via cellular composition.

The metabolic and fluxome data will be used to investigate and compare the physiological states of the different mutant strains with modified enzymes and pathways and elucidate the effect of the genetic perturbations on carbon flux and amino acids production.

References:

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