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Abstract title	13C DYNAMIC FLUX IDENTIFICATION: S. CEREVISIAE CULTIVATION UNDER FAST FEAST/FAMINE CONDITIONS
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Background

Understanding of metabolic robustness and regulation from system biology approaches will allow for more design-based metabolic engineering strategies. Three major challenges need to be tackled for in-vivo kinetic identification: (1) measurement limitations: the available data set contains only few observations of noisy data; (2) distinctive experimental setup: a high excitation is required and (3) parameter identification limitations, since kinetic models are highly non-linear.

Aims

To accurately estimate carbon fluxes under metabolic dynamic conditions from 13C labeling experiments and develop a kinetic model to describe the interaction of storage metabolism with central carbon metabolism.

Methods

An experimental platform based on a dynamic, cyclic feeding regime (20s feed in 400s cycle) is used to accommodate for concentration and labeling measurements. The cycles are highly reproducible, enabling to sample one cycle to obtain concentrations, switch to labeled substrate and follow the labeling enrichment. The concentration and labeling information is then evaluated to estimate dynamic flux profiles using a novel modeling approach [1]. The dynamic flux profiles are compared to those obtained under continuous cultivation conditions (aerobic culture of *S. cerevisiae* at an average growth rate of 0.1h-1).

Results

Measured time profiles for O2 and CO2 concentrations in the off gas and dissolved oxygen along with the feeding pattern prove a high reproducibility of the cycles. Time profiles for concentrations and enrichments of metabolites from glycolysis, pentose phosphate pathway and storage evidence the dynamic behavior of the complete metabolism during the cycle.

Comparing dynamic and steady-state conditions, shifts in metabolic activity are observed. Especially, the Pentose phosphate pathway seems to be more active under dynamic conditions. Under dynamic conditions, about 80% of the carbon uptake is entering glycolysis, 10% storage and 10 % is directed to the oxidative PPP. A slight decrease in biomass yield (5 %) has been observed.

Conclusions

The experimental approach allows for concentration and labeling measurements under dynamic conditions and together with a hybrid modeling approach allows for the estimation of short term flux profiles (400s). Dynamic metabolic fluxes can be reconstructed in time. Together with the measured concentrations, a start point for a further reconstruction of kinetic functions is set. New insight on the storage carbohydrate dynamics and its relation with central carbon metabolism have been obtained showing that under dynamic conditions the storage metabolism (glycogen and trehalose) seem to reduce the dynamics of flux changes.

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