

Automated and miniaturized ^{13}C-isotopic labeling experiments at microtiter plate-scale

JÜLICH Forschungszentrum

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Introduction: Principle of isotopic labeling experiments

1) Cultivation:

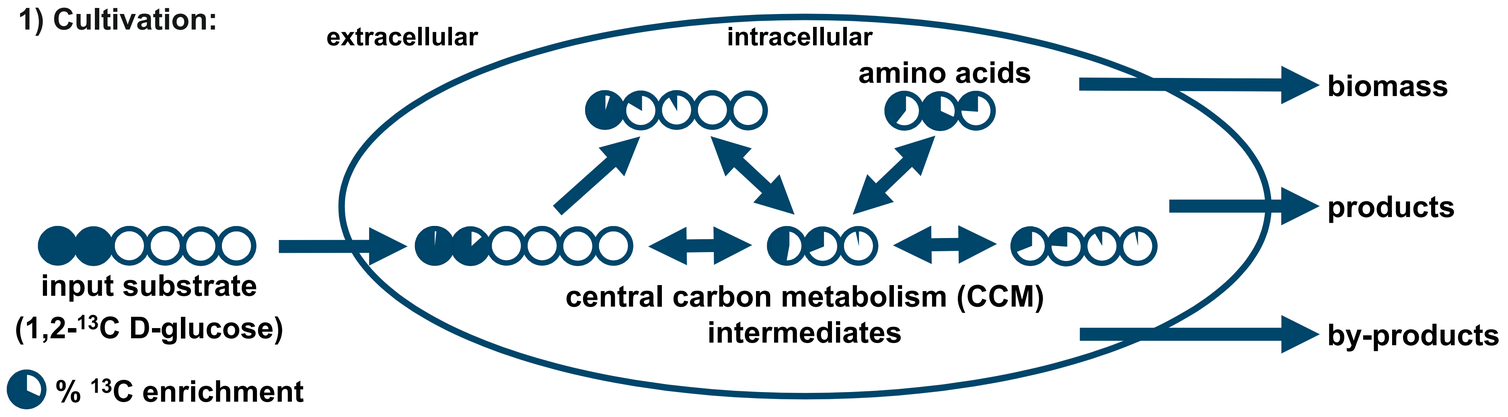
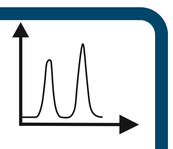


Fig. 1: Isotopic labeling experiments are conducted by cultivating cells on substrate(s) enriched with heavy isotopes.

2) LC-MS/MS analysis of amino acids and CCM intermediates



mass trace	peak area [a.u.]		relative abundance [a.u.]
$M0_m0$	1360904	normalization →	0.54
$M1_m0$	274559		0.11
$M1_m1$	687238		0.27
$M2_m1$	159080		0.06
$M2_m2$	43687		0.02
$M3_m2$	0		0

M(i) = mass of intact molecule + i labeled atoms
m(j) = mass of fragment + j labeled atoms

Fig. 2: Calculation of tandem mass isotopomer distributions.

Automated workflow based on hot isopropanol quenching

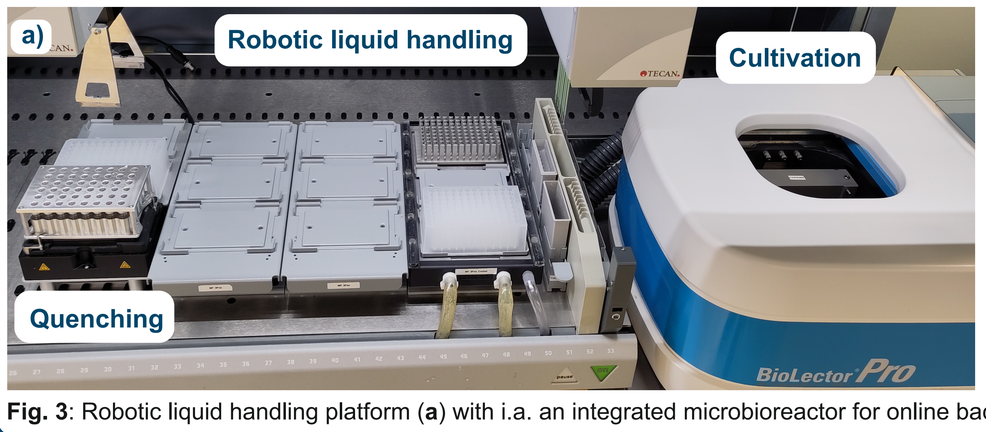
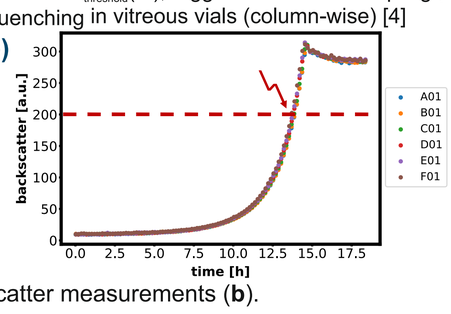


Fig. 3: Robotic liquid handling platform (**a**) with i.a. an integrated microbioreactor for online backscatter measurements (**b**).

Loop during BioLector cultivation:

- parsing and plotting online data (backscatter (BS), pH, dissolved oxygen) [1,2], then forwarding to experimenter
- if BS > $BS_{threshold}$(**–**), trigger automated sampling [3] and quenching in vitreous vials (column-wise) [4]



Automated hot isopropanol quenching:

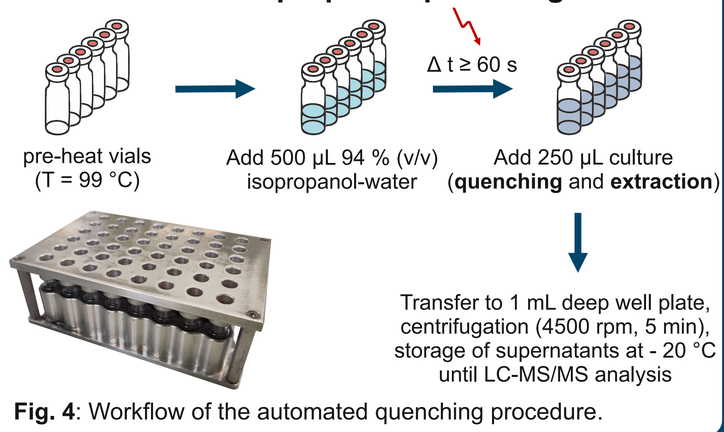


Fig. 4: Workflow of the automated quenching procedure.

Proof of concept: automated INST labeling experiment

- BioLector cultivation of *Corynebacterium glutamicum* ATCC13032 on CGXII medium [5] with 20 g/L U-^{12}C D-glucose
- BioLector settings: **$V_{L,start}$ = 750 µL**, n = 1400 rpm, T = 30 °C
- Backscatter-triggered **pulse** (**50 µL DDW with 80 g/L U-^{13}C D-glucose**) with subsequent automated sampling and hot isopropanol quenching at staggered time points (fastest time point ~ 24.5 s after pulse)

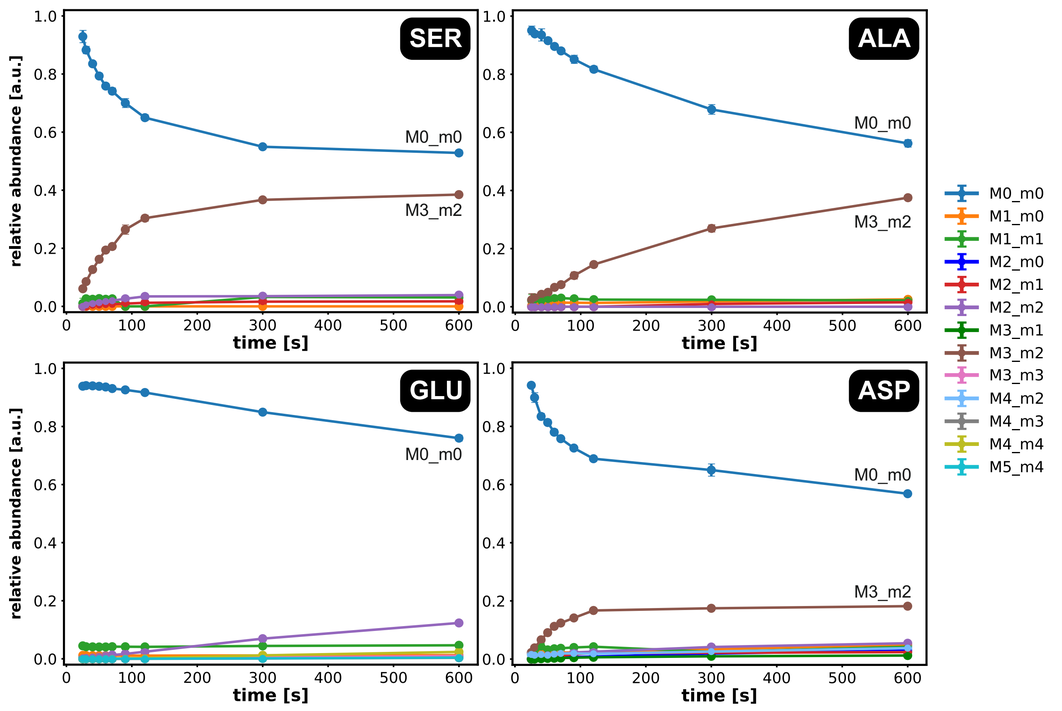


Fig. 5: Time courses of relative mass trace abundances of amino acid fragments. As an excerpt, a fragment of L-serine (m/z = 60), L-alanine (m/z = 44), L-glutamate (m/z = 84) and L-aspartate (m/z = 74) are presented.

➡ The speed of the robotic workflow was demonstrated to be sufficient for generating **informative dynamic labeling data of free amino acids**.

Pipeline for bioprocess modelling

- Define differential algebraic system of equations in OpenModelica [6]
- Allows easy definition of state events (pulsing, fed-batch etc.)

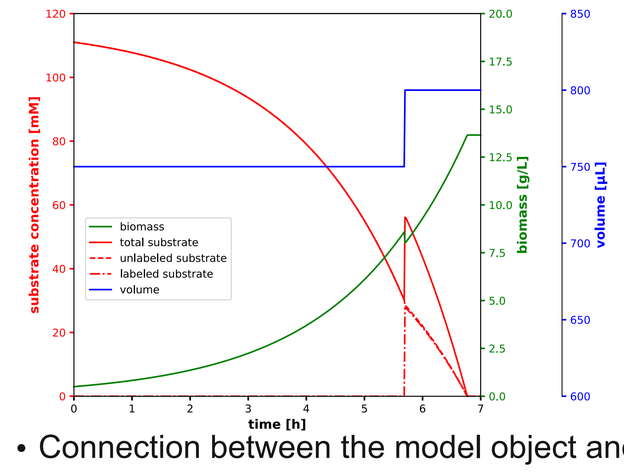


Fig. 6: Exemplary plot portraying parameters of a bioprocess model for an INST labeling experiment with a pulse of labeled substrate during the exponential growth phase. Particularly, the influence of the pulse on the biomass and substrate concentrations can be observed.

- Connection between the model object and the in-house developed package hopsy [7] (Python interface to the sampling library HOPS [8]) for uncertainty quantification on a cluster

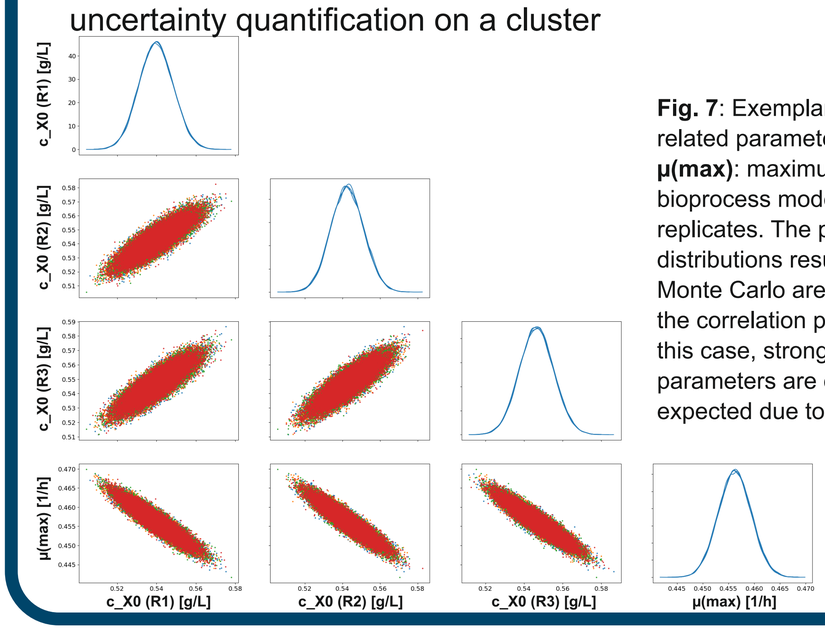


Fig. 7: Exemplary pair plot of biomass related parameters (**c_X0**: initial biomass, **µ(max)**: maximum growth rate) from a bioprocess model with three biological replicates. The posterior probability distributions resulting from Markov chain Monte Carlo are shown as blue lines and the correlation plots as point clouds. In this case, strong correlations between the parameters are observed which is expected due to the model formulation.

References:

[1] Osthege, M., et al., *bletl - A Python package for integration microbioreactos in the design-build-test-learn cycle.* bioRxiv, 2021.

[2] Michael Osthege, Niklas Tenhaef, Laura Helleckes, Jochen Nießer, Lars Halle, & Carolin Müller. (2022). JuBiotech/bletl: v1.1.3 (v1.1.3). Zenodo. https://doi.org/10.5281/zenodo.6884009

[3] Michael Osthege, Laura Helleckes, Lars Halle, Jochen Nießer, Stephan Noack, Clay Kosonocky, Carolin Müller, & Valentin Steier. (2023). JuBiotech/robotools: v1.5.3 (v1.5.3). Zenodo. https://doi.org/10.5281/zenodo.7760294

[4] Niesser, J., et al., *Hot isopropanol quenching procedure for automated microtiter plate scale ^{13}C-labeling experiments.* Microb Cell Fact, 2022. 21(1): p. 78.

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[6] Fritzson, P., et al., *The OpenModelica Integrated Environment for Modeling, Simulation, and Model-Based Development*, MIC Journal, 2020, 41(4): p. 241-295.

[7] Paul, R., Jadebeck, J. F., https://github.com/modsim/hopsy.

[8] Jadebeck, J. F., Theorell, A., Leweke, S., Nöh, K., *HOPS: high-performance library for (non-) uniform sampling of convex-constrained models*, Bioinformatics, 2021, 37(12): p. 1776-1777.

Conclusion

- **Proof of concept:** using the automated workflow, an **INST labeling experiment** with the model organism *Corynebacterium glutamicum* ATCC13032 was conducted.
- **The automated workflow** enables access to **isotopically stationary and instationary labeling data of intracellular amino acids and CCM intermediates** via experiments **at a microtiter plate-scale** in **48 parallel batches, a reduced time frame** (proof of concept: isotopic labeling experiment in one work day) and **vastly decreased substrate costs**.
- Pipeline for **bioprocess modelling** with **uncertainty quantification** was set up.