Rapid determination of *E. coli* growth kinetic parameters in a 96-well microplate using a new online monitoring system

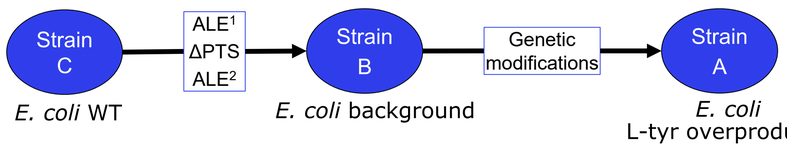
Mykhaylo Semenov Petrov[1], Laura Civil Ferrer[1], Ivan Schlembach[1], Andreas Schulte[2], Juan Camilo Porras Correa[2], Lei Yang[1], Tibor Anderlei[3], Suresh Sudarsan[1]

1) DTU Biosustain, DK, 2) Kuhner Shaker, DE 3) Adolf Kühner AG, CH

The rational development of aerobic cell factory-based processes necessitates a comprehensive understanding of substrate and oxygen uptake kinetics. Conventional methods, such as substrate-limited chemostats or constant-feed fed-batch experiments, can be employed to determine kinetic parameters but can rather be labor-intensive, highlighting the need for a prompt approach to assess such parameters on a micro-scale cultivation level.

In this study, we utilized the state-of-the-art µ-scale cultivation device (Kuhner microTOM), determining in each well of a 96 deep well microplate the oxygen transfer rate curve.

To demonstrate the efficiency of the Kuhner microTOM System a tyrosine-producing *Escherichia coli* strain (A) was characterized and compared with its background (B), and a WT (C) strains (for Ks and OTR_{max} determination).



Kuhner microTOM

Experiment 1

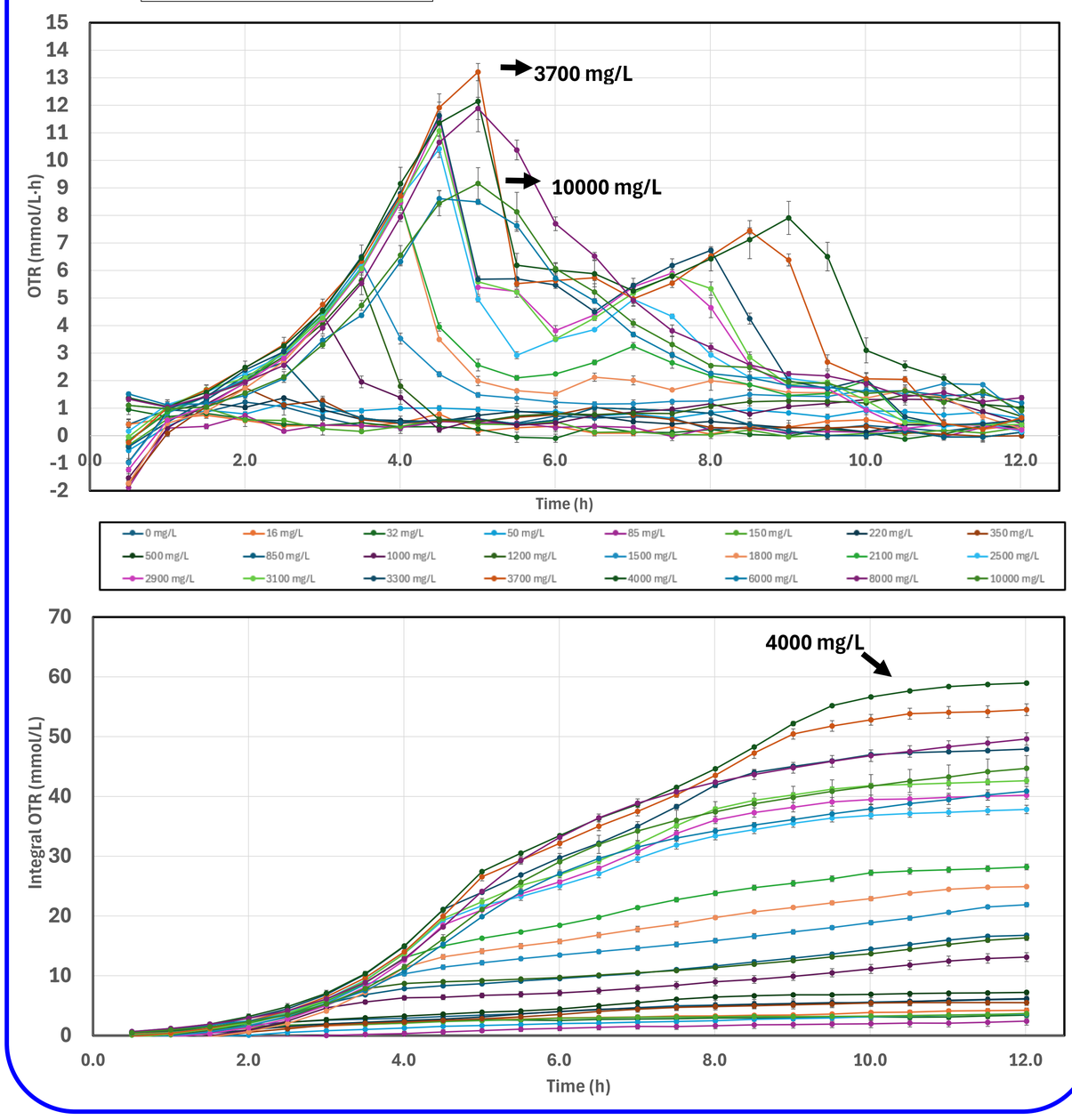
O/N culture in **M9** minimal medium using micro**TOM** → Measure cell growth (OD_{600})

Dilute to initial OD_{600} = 0.1 using M9 medium with **24 glucose concentrations**
Ranging from 0 to 10.000 mg/L
(24 conditions x 4 replicates each)

Record **OTR** and **integral OTR** using microTOM

Estimation of biomass, O_2 yield coefficients ($Y_{X/S}$, $Y_{O/S}$, $Y_{O/X}$) and growth rate (µ)

Cultivation conditions	
Shaking diameter:	50 mm
Shaking speed:	225 RPM
Cultivation volume:	300 µL



Experiment 2

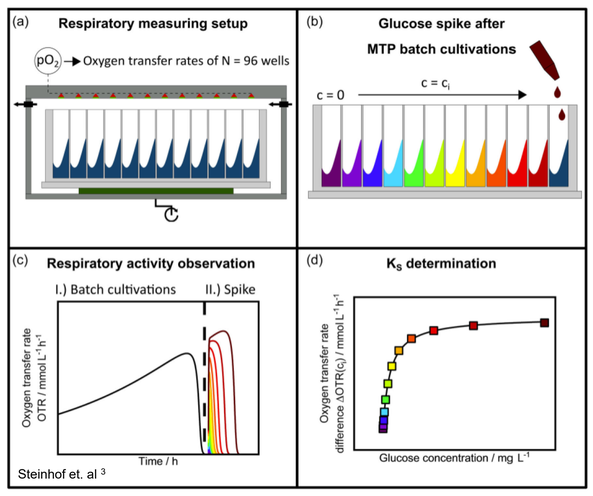
O/N culture in **M9** seed train medium with **1$g_{glucose}$/L** using micro**TOM** → Measure cell growth (OD_{600}); aliquot in 96-well plate

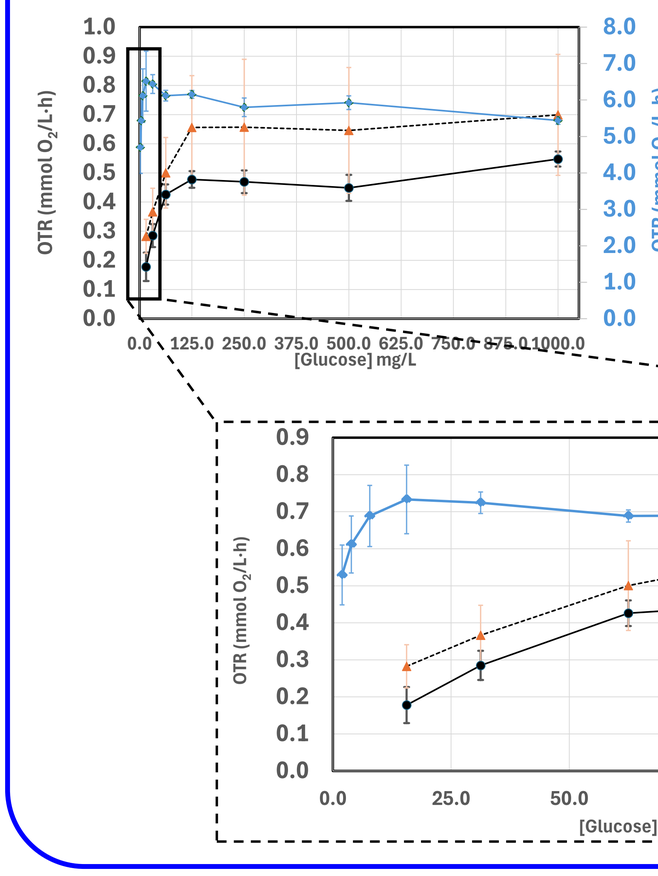
Let cells undergo **glucose starvation**

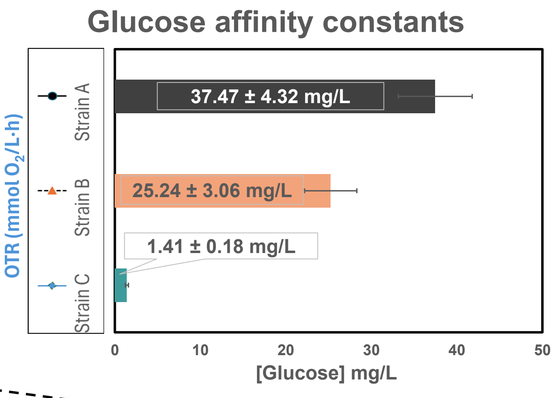
Spike glucose solution to reach **different final concentrations** (0-2000 mg/L)

Record **pO_2** (OTR) using microTOM

Estimation of Ks for *E. coli* overproducing (**A**), background (**B**) and WT (**C**) strains







- **Maximum growth rate (μ_{max}):** The usable data were those conditions that remained stable and free of noise, which corresponded to glucose levels well above the Ks of strain A. A maximum growth rate of **μ_{max} = 0.70 ± 0.01 h^{-1}** was determined.. As a result, the values reflect growth under saturating conditions, and a separate experimental setup would be required to link OTR with µ values at lower, sub-saturating glucose concentrations where growth becomes limited.

- **Yield coefficients:** Based on start and end OD_{600} values, the following yields were calculated for strain A: **$Y_{O/S}$ = 0.21 ± 0.01**, **$Y_{X/S}$ = 0.28 ± 0.01**, and **$Y_{O/X}$ = 0.76 ± 0.02**. The biomass yield ($Y_{X/S}$) is lower than the literature value (0.45)[4], reflecting carbon redirection toward L-tyrosine and by-products caused by ΔPTS. Likewise, the oxygen-to-biomass ratio ($Y_{O/X}$) is reduced compared to literature (0.945)[5], indicating an altered respiratory metabolism with less oxygen consumed per biomass formed.

- **Strain physiological implications:** Strain A exhibits a redistribution of carbon flux, with more substrate directed toward L-tyrosine synthesis at the expense of biomass accumulation and energy generation

- **Lower $Y_{X/S}$ and $Y_{O/X}$** values compared to literature[4,5] are observed, confirming that growth efficiency is reduced in favor of product formation.

- **Use of Kuhner microTOM**: the device proved effective to determine essential microbial kinetic parameters in early process development stages. It allowed accurate measurement of OTR, µmax, and yield coefficients under well-controlled conditions. Its ability to deliver high-resolution data confirms it as a practical and fast tool for characterizing and comparing production strains in small-scale experiments.

References

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Adolf Kühner AG
Dinkelbergstrasse 1
CH-4127 Birsfelden, Switzerland
+41 (0)61 319 93 93, www.kuhner.com