

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

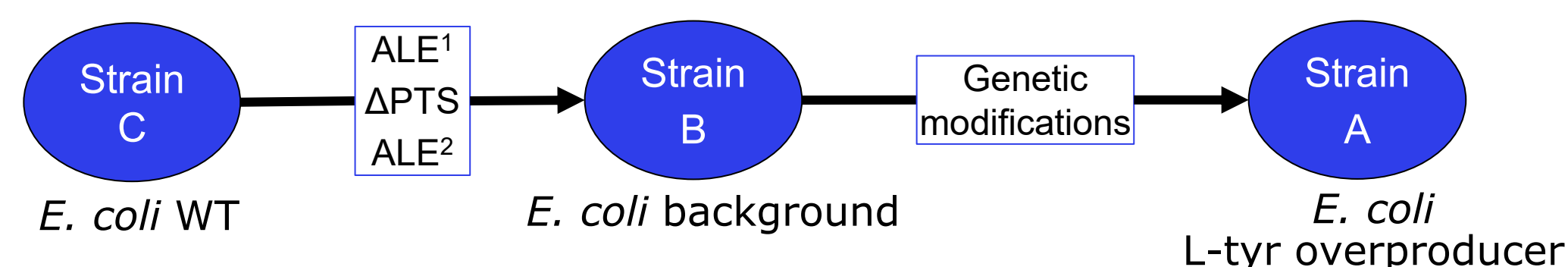
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The rational development of aerobic cell factory-based processes necessitates a comprehensive understanding of substrate and oxygen uptake kinetics. Conventional methods, such as substrate-limited chemostats or constant-feed fed-batch experiments, 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 K_s and OTR_{max} determination).



Experiment 1

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

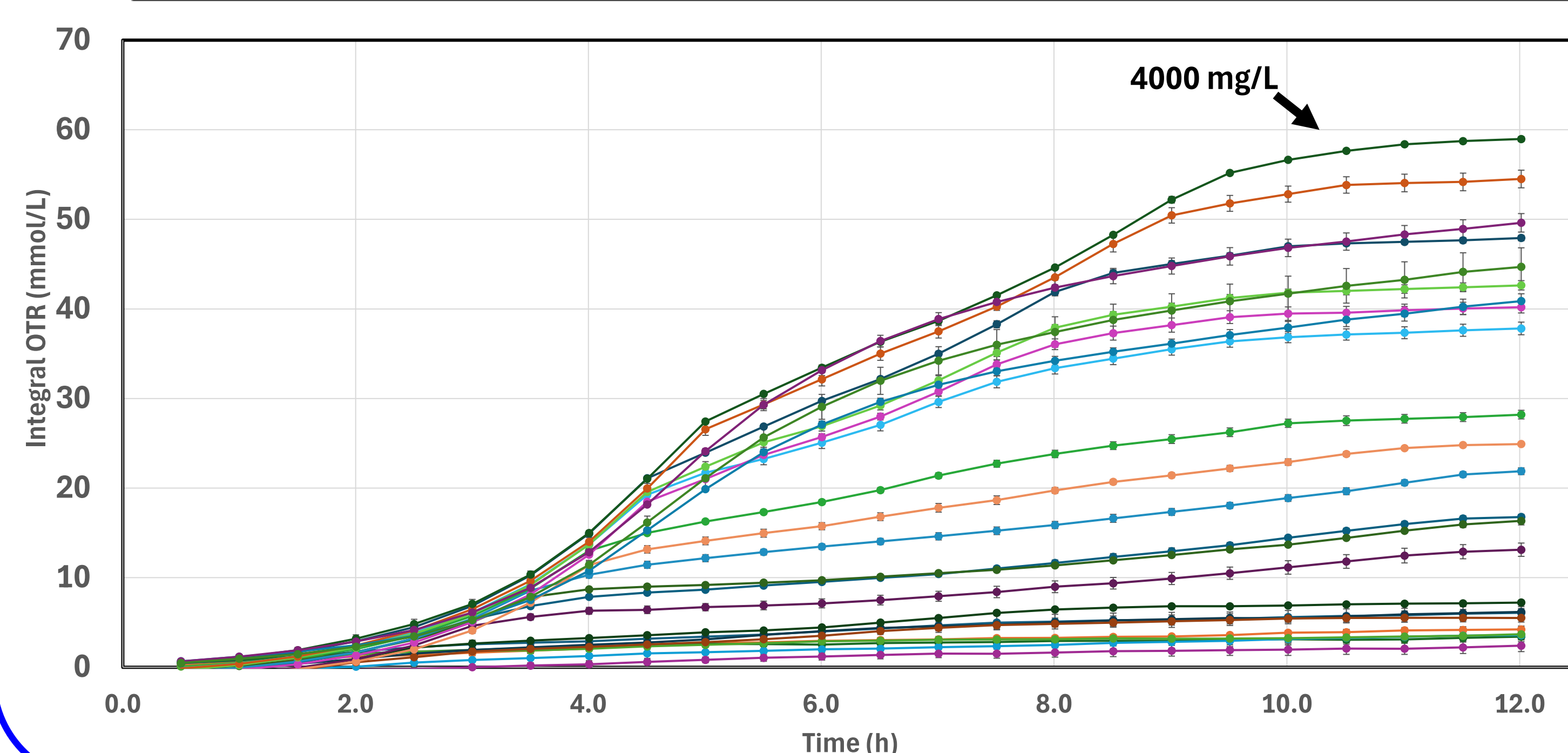
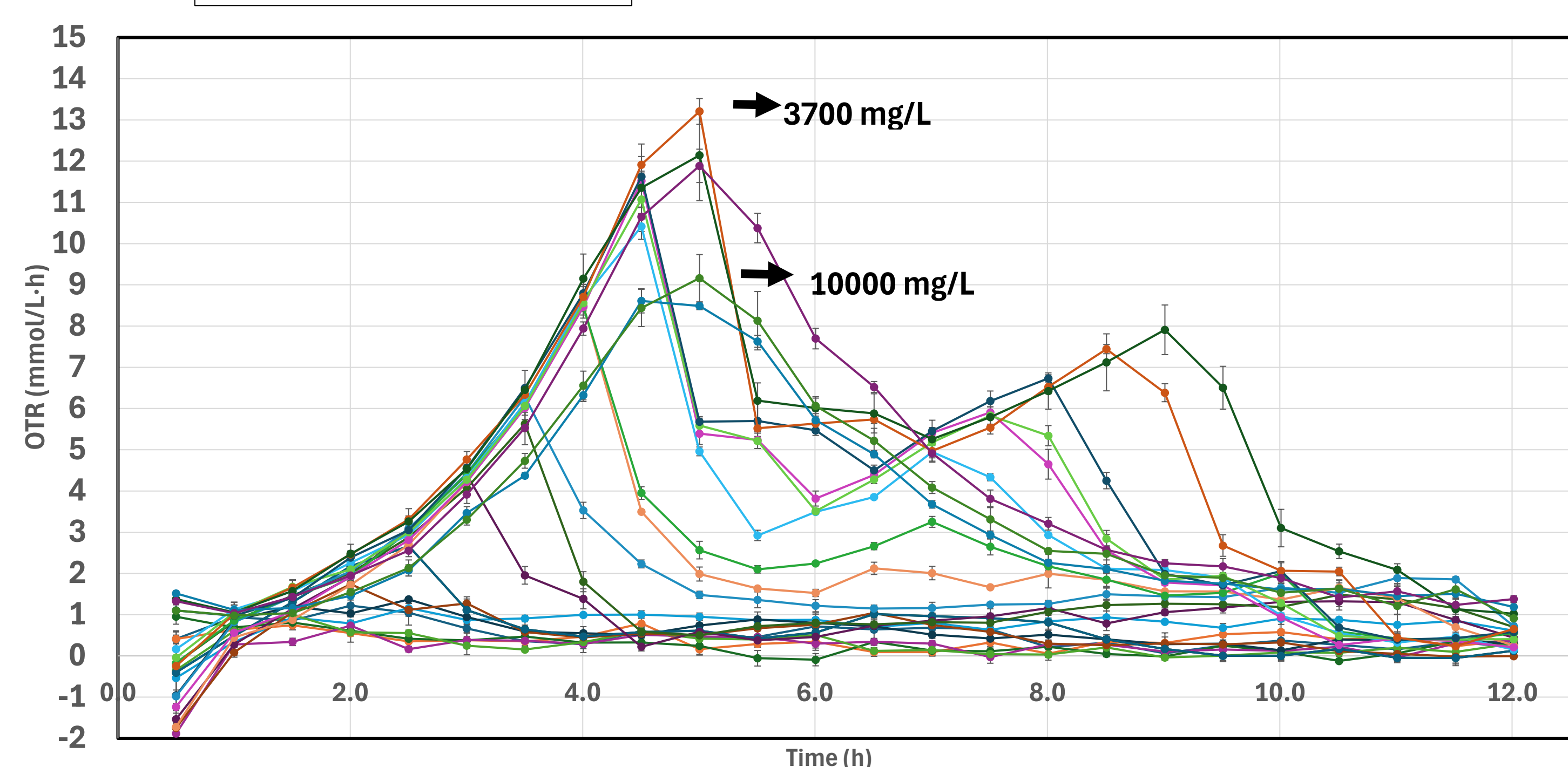
O/N culture in M9 minimal medium using microTOM

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

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



- 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 K_s of strain A. A maximum growth rate of $\mu_{max} = 0.70 \pm 0.01 \text{ h}^{-1}$ 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: $Y_{O/S} = 0.21 \pm 0.01$, $Y_{X/S} = 0.28 \pm 0.01$, and $Y_{O/X} = 0.76 \pm 0.02$. 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 Δ P_TS. 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.



Experiment 2

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

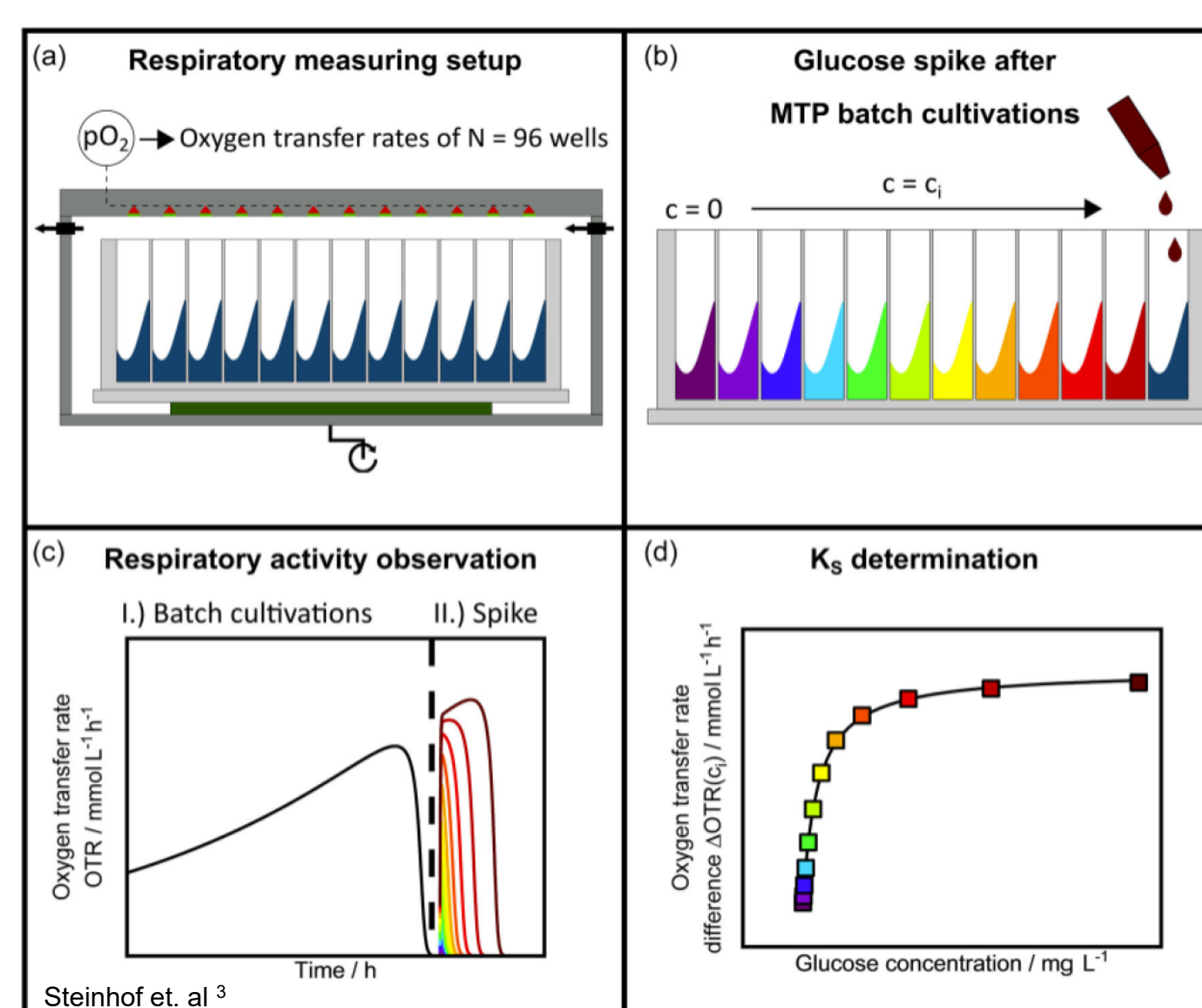
O/N culture in M9 seed train medium with 1g glucose/L using microTOM

Measure cell growth (OD_{600}); aliquot in 96-well plate

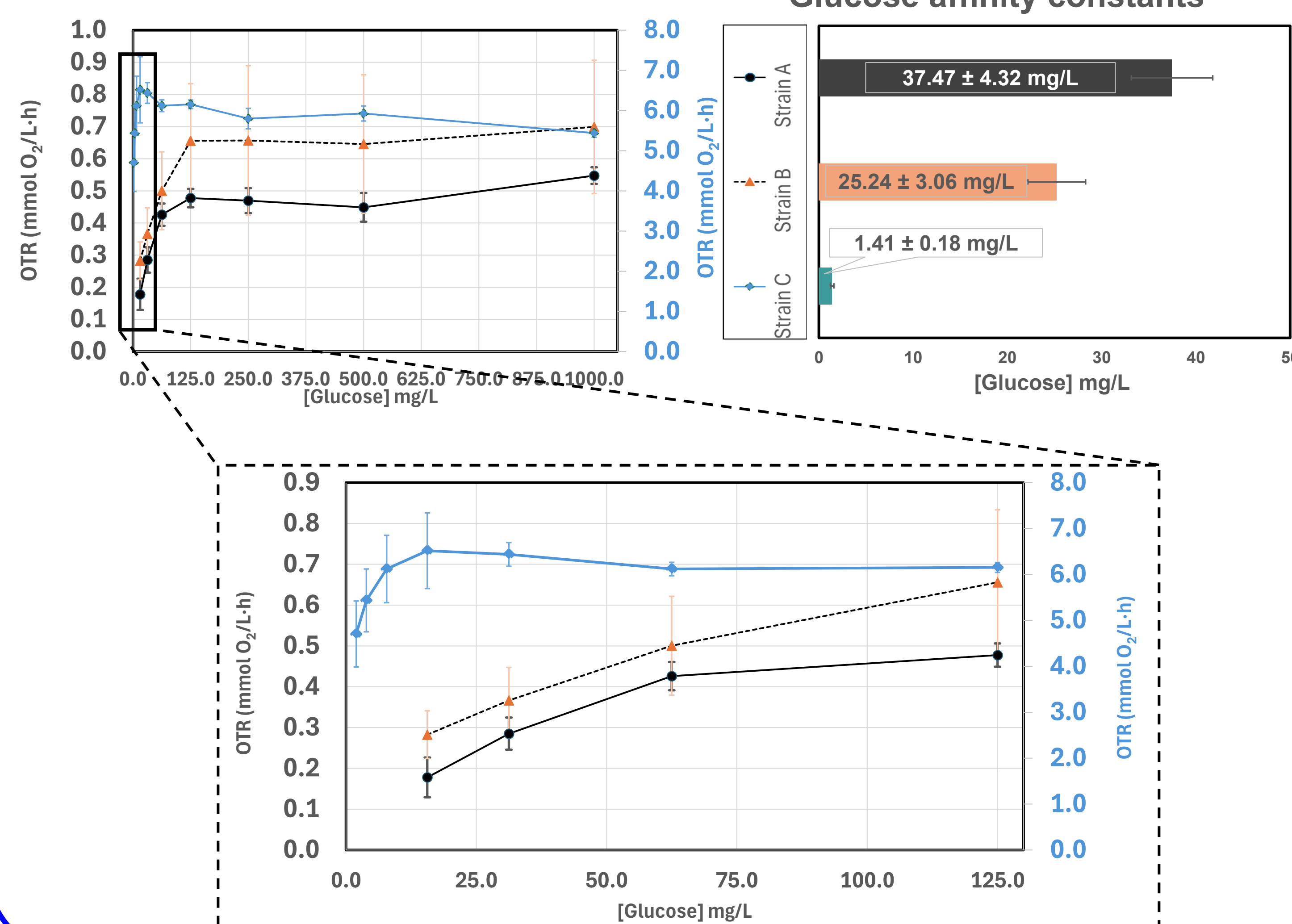
Let cells undergo glucose starvation

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

Record pO_2 (OTR) using microTOM



Glucose affinity constants



- 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, μ_{max} , 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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