

# Early Bioprocess Development using *Komagataella phaffii*: Application Scenarios for the microTOM Mini Bioreactor System

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## Introduction

Early bioprocess development relies on high-throughput systems like 96-deepwell microtiter plates (MTPs) to screen strains and conditions. The **microTOM** (micro scale Transfer rate Online Measurement) measures **Oxygen Transfer Rate (OTR)** in each individual well of a MTP [1]. As a direct indicator of metabolic activity, OTR is essential for identifying growth behaviour, substrate consumption and oxygen uptake and limitations. Using *Komagataella phaffii* with varying methanol utilization phenotypes (BG10 (Mut<sup>+</sup>), X-33 (Mut<sup>+</sup>) and CS310 (Mut<sup>S</sup>)) as model organisms, we demonstrate the system's versatility in screening for strain and cultivation conditions.

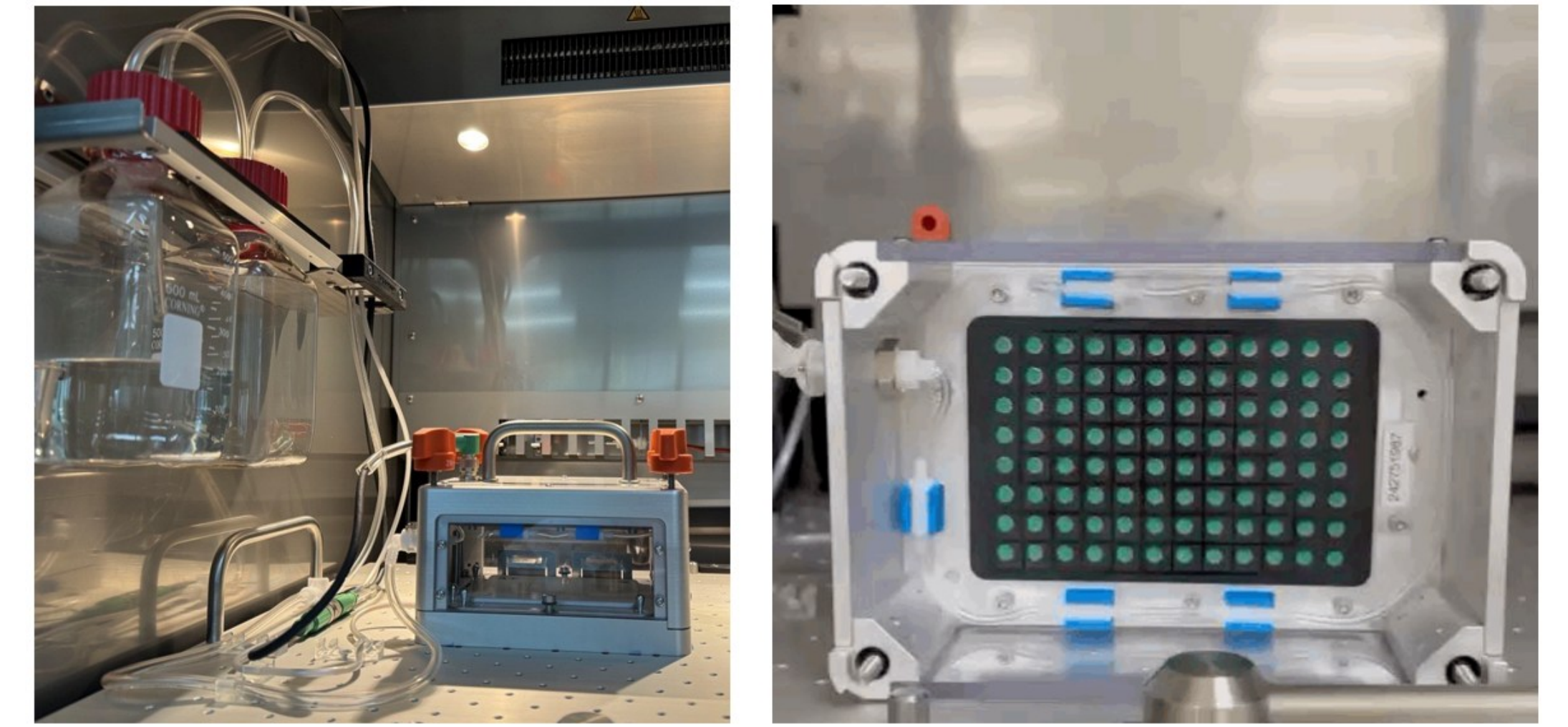


Figure 1: microTOM device for OTR measurement in 96-well MTPs

## Media Screening

All three *K. phaffii* strains were tested on two carbon sources (glucose and glycerol) in four different YP media (varying the yeast extract source) and two defined mineral media (Syn6-MES and Seed). A single 96-well MTP run was sufficient to compare all conditions with N=4 replicates per condition.

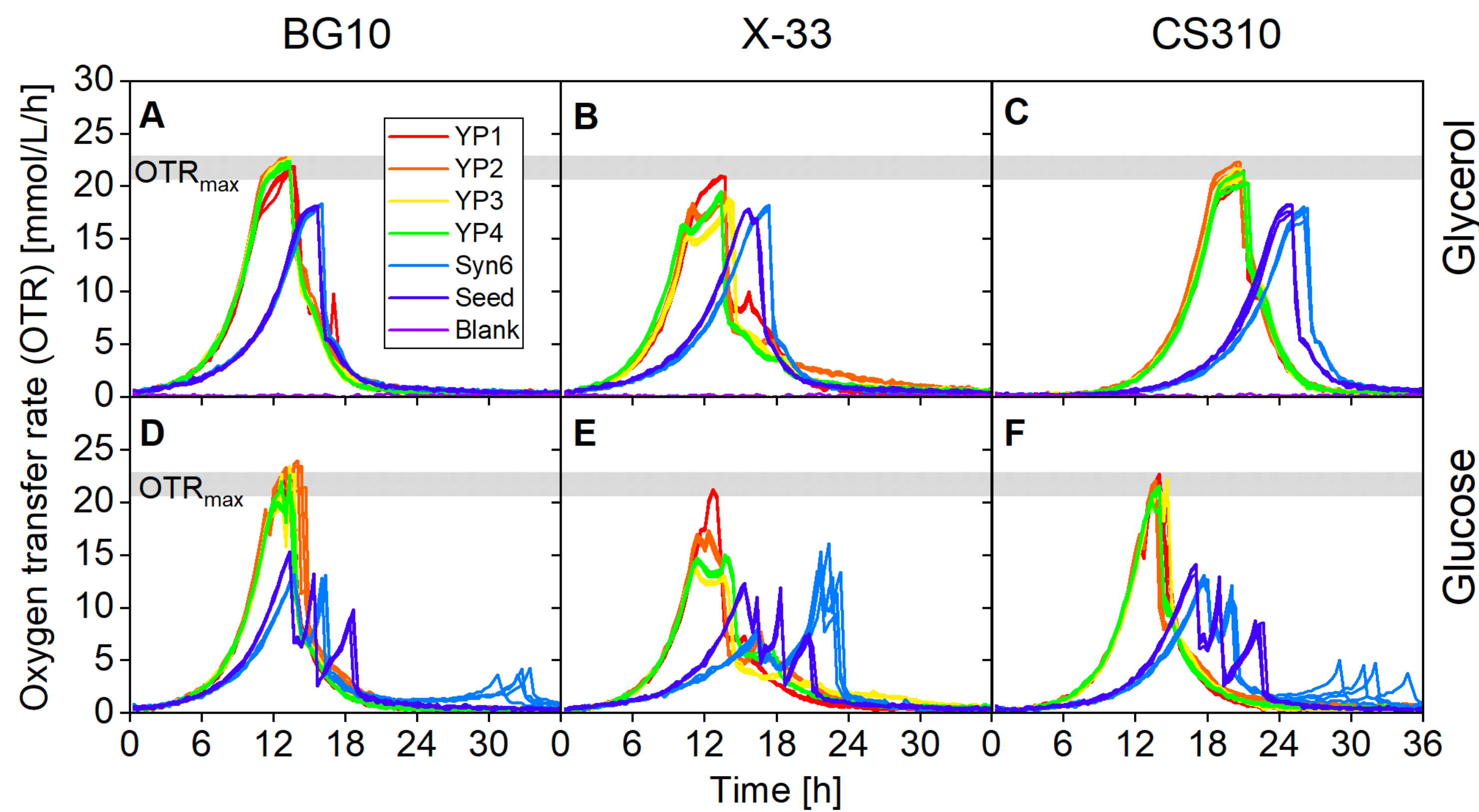


Figure 2: OTR of three *K. phaffii* strains on glycerol (top) and glucose (bottom) in YP media (4 yeast extracts) and mineral media (Syn6-MES, Seed). 96-well MTP, 0.6 mL, 300 rpm, 50 mm, 28 °C, N=4

- BG10 and CS310 showed no OTR differences between the tested yeast extracts on glycerol
- Strain X-33 exhibited differences between yeast extract sources, with double peaks visible for some formulations
- On glucose, additional OTR peaks after 16–30 h indicate overflow metabolite formation (e.g. ethanol, acetate)
- Mineral media caused delayed initial growth compared to YPD, and the two mineral media showed distinct metabolic patterns on glucose

## Temperature Screening

The OTR signal is proportional to biomass and can be used to estimate the specific growth rate. Three strains were cultivated at 20, 25, 28, 32, 34, and 37 °C on mineral Seed medium with 10 g/L glycerol. The estimated growth rate was plotted against temperature, resulting in a bell-shaped response curve.

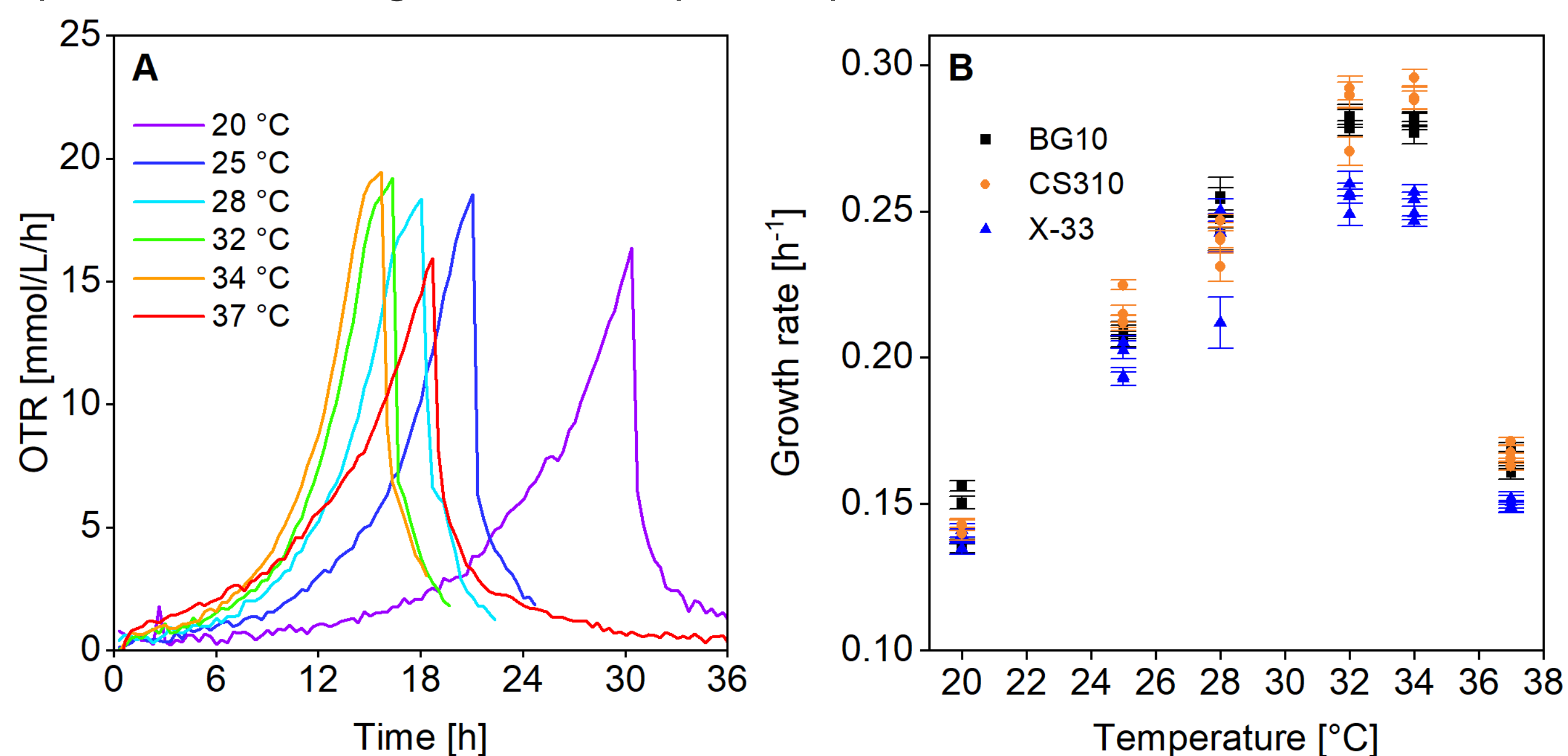


Figure 3: A) OTR for *K. phaffii* BG10 at different cultivation temperatures (1 of 4 replicates). B) Growth rate estimated from OTR plotted against temperature for all three strains. Seed medium, 10 g/L glycerol, 0.6 mL, 300 rpm, 50 mm, N=4.

- Optimal growth temperature was 32–34 °C for BG10 and CS310
- Growth rate plateau for X-33 from 28–34 °C and overall slower growth
- Rapid temperature profiling for clone libraries without offline measurements

## Methanol Uptake Rate Estimation

*K. phaffii* is a methylotrophic yeast with strong methanol-inducible promoters. A common challenge is determining the appropriate methanol feeding rate for fed-batch processes. After initial growth on glycerol, 1% v/v methanol was added for adaptation, followed by **spiking of 0.5–3.5% v/v methanol**. The time required for complete consumption ( $\Delta t$ ) was used to estimate the uptake rate.

Figure 4: OTR at methanol consumption for A) BG10 Mut<sup>+</sup> and B) CS310 Mut<sup>S</sup>. Phases: I) glycerol, II) MeOH adaptation, III) MeOH consumption, IV) depletion. Seed medium, 0.6 mL, 300 rpm, 50 mm, 30 °C, N=4.

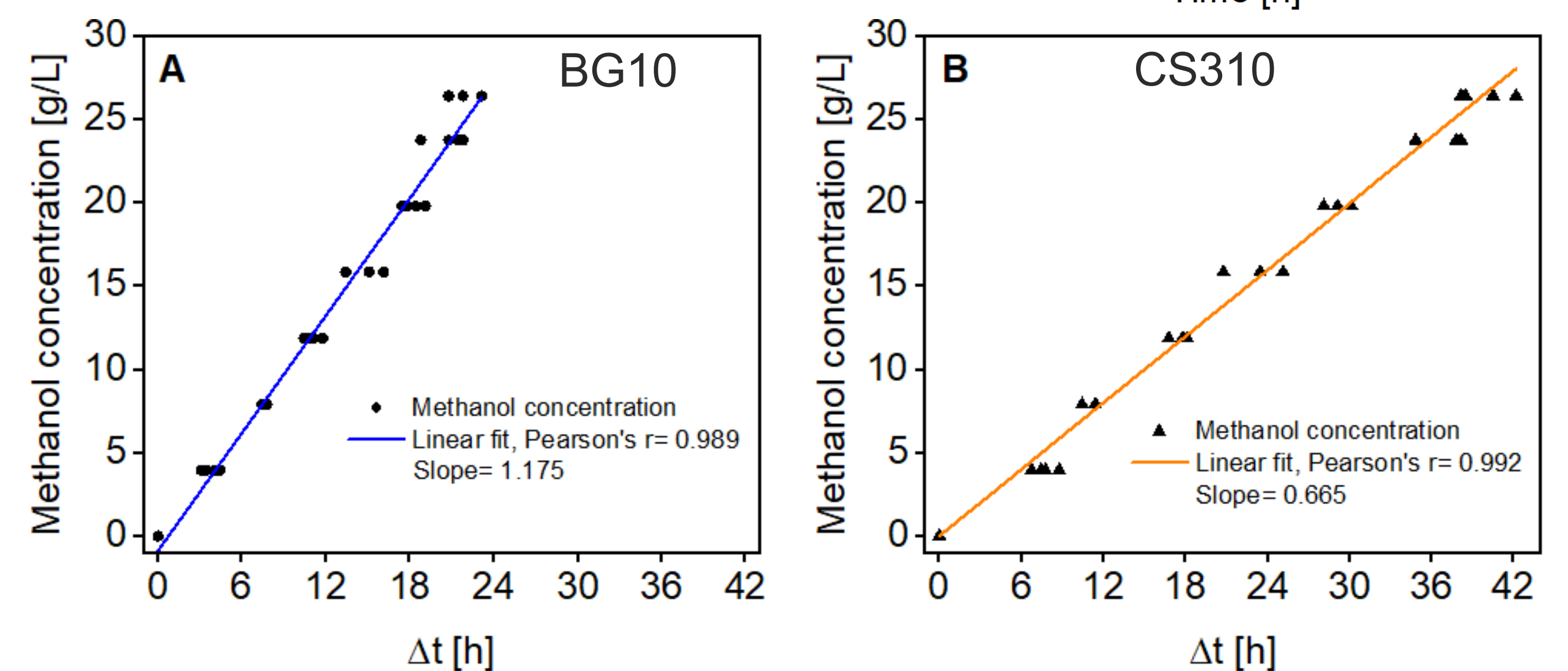
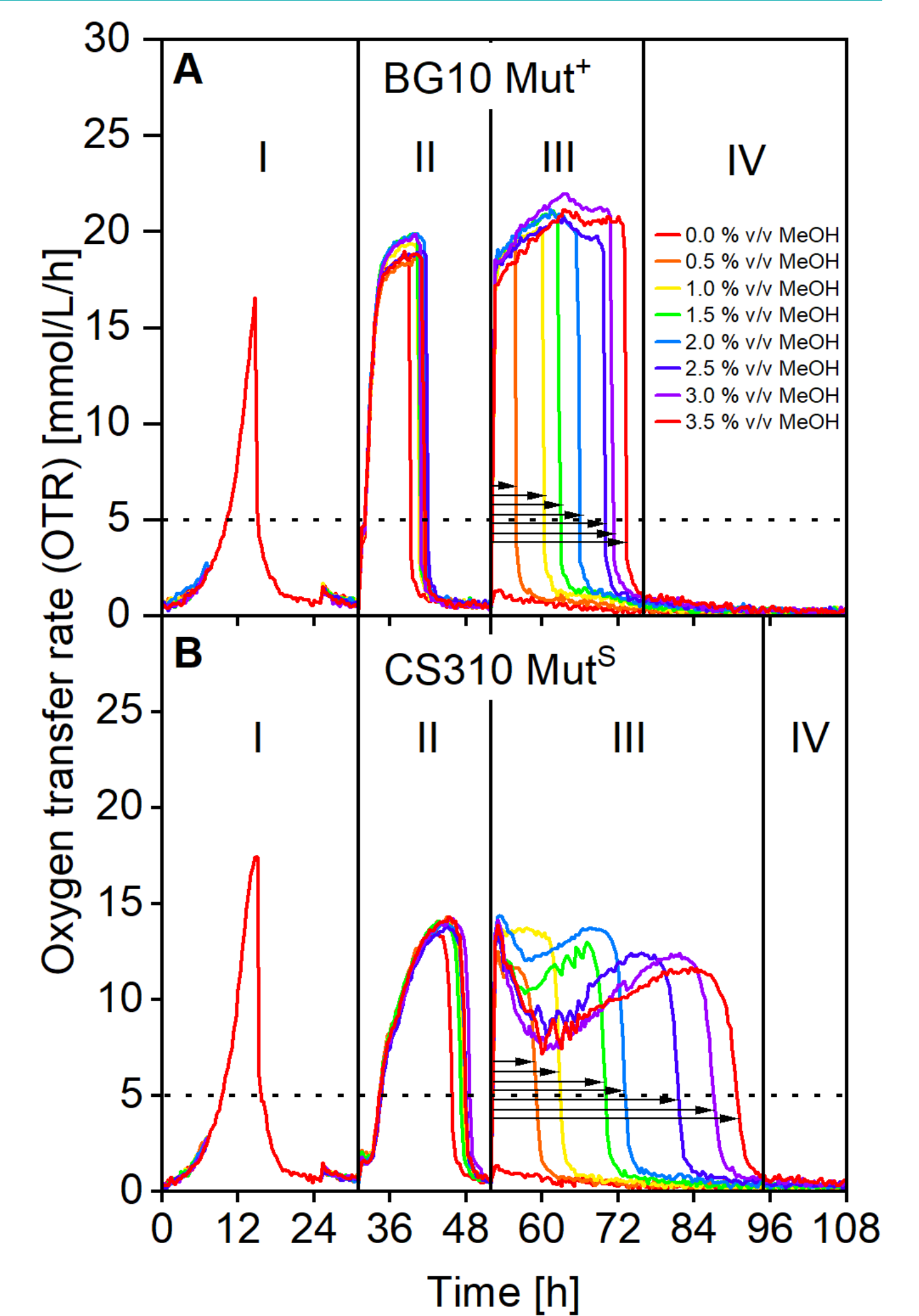


Figure 5: Methanol uptake rate. Linear fit of MeOH concentration vs.  $\Delta t$  for BG10 Mut<sup>+</sup> (1.175 g/L/h) and CS310 Mut<sup>S</sup> (0.665 g/L/h).

- The Mut<sup>+</sup> strain BG10 showed methanol uptake rates of 0.21, while the Mut<sup>S</sup> strain CS310 showed 0.11 g/g/h
- Values are consistent with literature [2]
- Rapid estimation without the need for HPLC analysis and can guide feeding rates for fed-batch processes

## Conclusion

- Successful application of microTOM to multiple scenarios of *K. phaffii* at 96-well MTP scale
- OTR measurements revealed differences between **media compositions**, yeast extract sources, and carbon sources
- **Temperature screening** identified growth optima of 32–34 °C for BG10 and CS310
- **Methanol uptake rates** were rapidly estimated without HPLC, providing values that can directly inform fed-batch feeding strategies
- **Data-driven bioprocess development** at high throughput with sufficient replicates for data robustness

### References

- 1 Dinger et al. (2022) Biotechnol. Bioeng. 119(3), 881–894
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- 3 Wollborn et al. (2022) Sci. Rep. 12, 11225
- 4 Krainer et al. (2012) Microb. Cell Fact. 11, 22