

Monitoring VCD, Glucose and Lactate using off-gas analysis and hybrid modeling

Kuhner shaker

Andreas Schulte¹, Tibor Anderlei^{1*}, Harini Narayanan², Jakub Polak², Lukas Arndt³, Ralf Pörtner³, Anna-Lena Heins³, Anna Christina Adams⁴, Anne Neuss⁴, Jørgen Barsett Magnus⁴

¹ Kuhner Shaker, Birsfelden, Switzerland *presenting author

² DataHow, Zürich, Switzerland

³ TUHH, Institute of BioProcess and Biosystems Engineering, Hamburg, Germany

⁴ RWTH Aachen University, Biochemical Engineering, Aachen, Germany

Introduction and Novelty

In modern bioprocess engineering, monitoring of biomass and key metabolites is essential for robust process control and quality assurance. In R&D this is commonly done via manual sampling and subsequent analysis. As invasive sampling increases the risk of contamination and provides delayed data, a soft-sensor technology based on off-gas analysis is presented to online predict VCD, Glucose and Lactate.

Off gas analysis was done in shake flasks to allow for medium throughput using a Kuhner TOM. Exemplary, a CHO DP12 cell line was cultivated in TCX-6D and TC-42 medium.



Oxygen transfer rate (OTR) and carbon dioxide transfer rate (CTR) as online indicator of cell behaviour

In the past 10 years, measurement of Oxygen transfer rate and Carbon dioxide transfer rate has been increasingly applied in mammalian cell cultivations on shake flask scale, achieving better process understanding and optimization in early-stage process development.

It has been shown in recent publications, that oxygen consumption and carbon dioxide production are strongly connected to the physiological state of the cells and can unveil a variety of phenomena:

- ✓ Cell growth and Viable cell count (VCD) [1,2,3,4]
- ✓ Carbon source consumption [1,3,5]
- ✓ Lactate formation and consumption [3]
- ✓ Glucose, Lactate, Glutamine depletion [1,3]
- ✓ Hydromechanical stress [1]
- ✓ Antibody production [1,4]

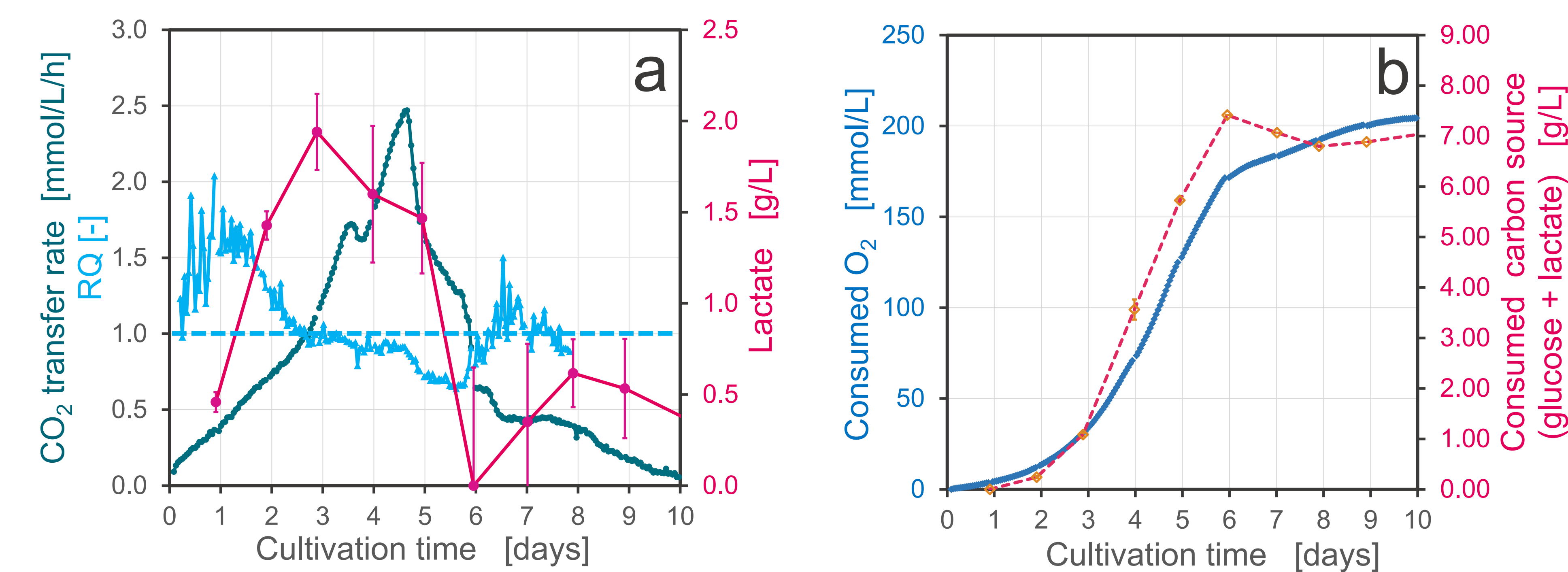


Figure 2: CHO DP12 cultivation in TCX-6D medium. Data derived from Figure 1
a) Dependency of RQ and Lactate metabolism b) Carbon source and O₂ consumption

Day 0-3 When lactate is formed, the respiratory quotient is high as pH decreases and the bicarbonate buffer releases CO₂

Day 6 When lactate is consumed the RQ falls below 1 as described by Ihling et al. [3].

General correlation between consumed carbon source and consumed oxygen. Oxygen is needed to metabolize glucose and lactate. Very low respiration after carbon depletion at **Day 6**.

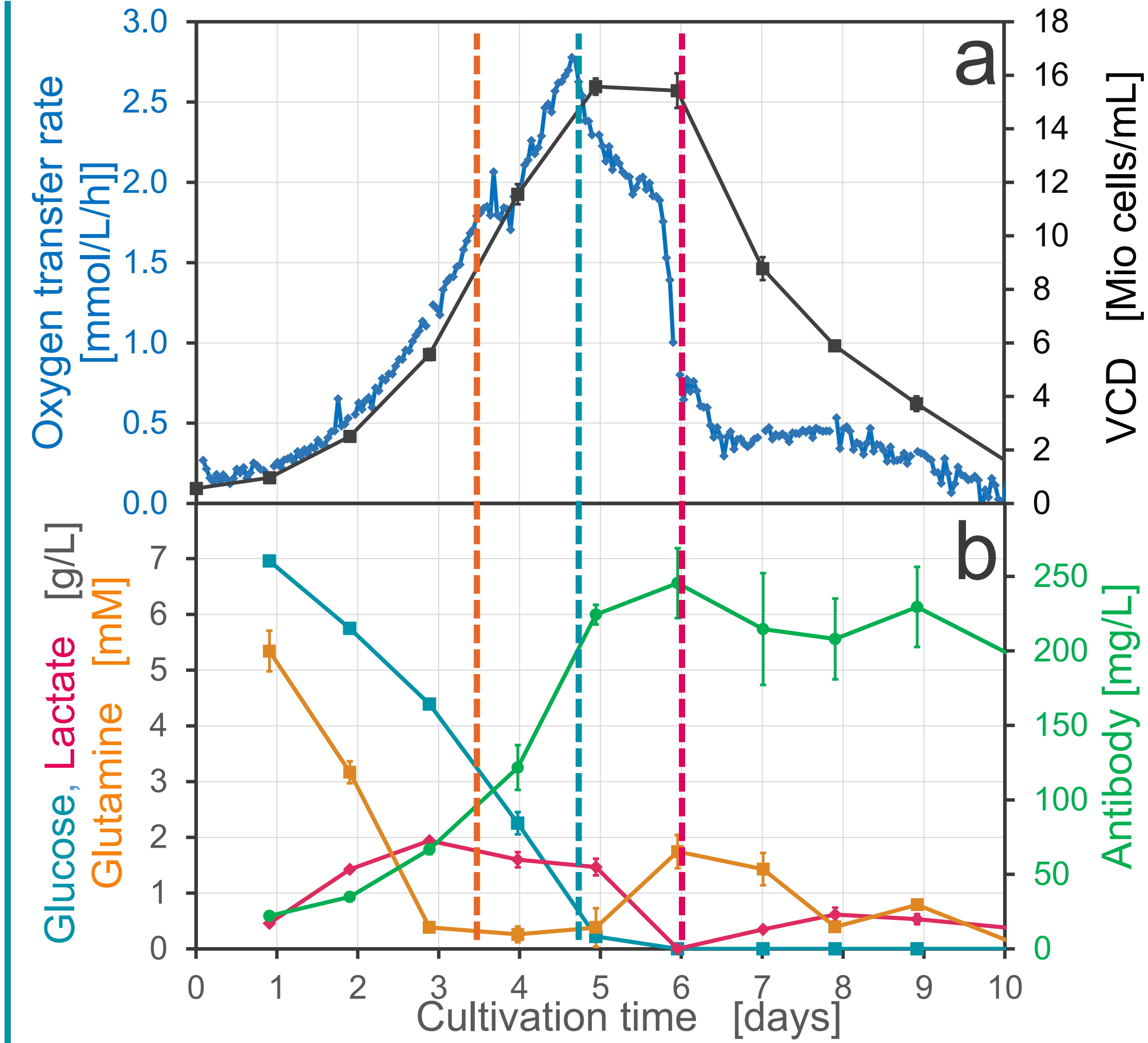


Figure 1: CHO DP12 cultivation in TCX-6D medium, 250mL shake flask. Correlation between Oxygen transfer rate, VCD and metabolite concentrations.

Figure 1 is a rearrangement of previously published data from Neuss et al. [1]. Viable cell count (upper graph) is well correlated to oxygen transfer. Distinct shoulders indicate Glutamine, Glucose and Lactate depletion (dashed lines). The end of antibody production can be observed when oxygen consumptions drops (after day 6).

Model predicted data from Off-gas analysis data

Contextual information

- Cell line
- Medium
- Process conditions
-



Training datasets related to context

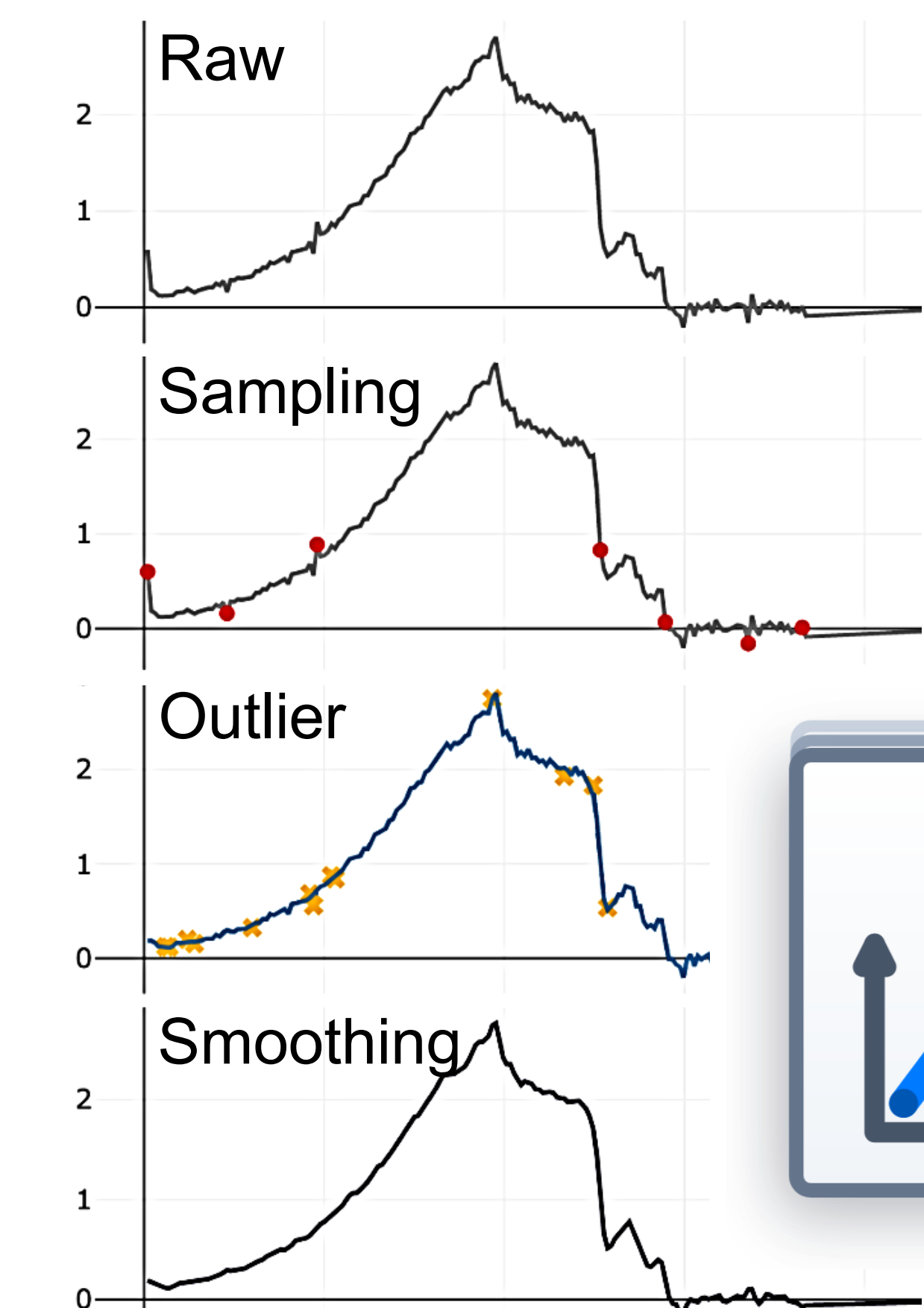


Figure 3: Training dataset CHO DP12, TCX-6D medium

Hybrid model

Hybrid models are a fusion of hard coded mechanistic models, which code known process dynamics, with machine learning models that provide insight into what is unknown from provided data.

Online off-gas data (OTR, CTR, RQ)

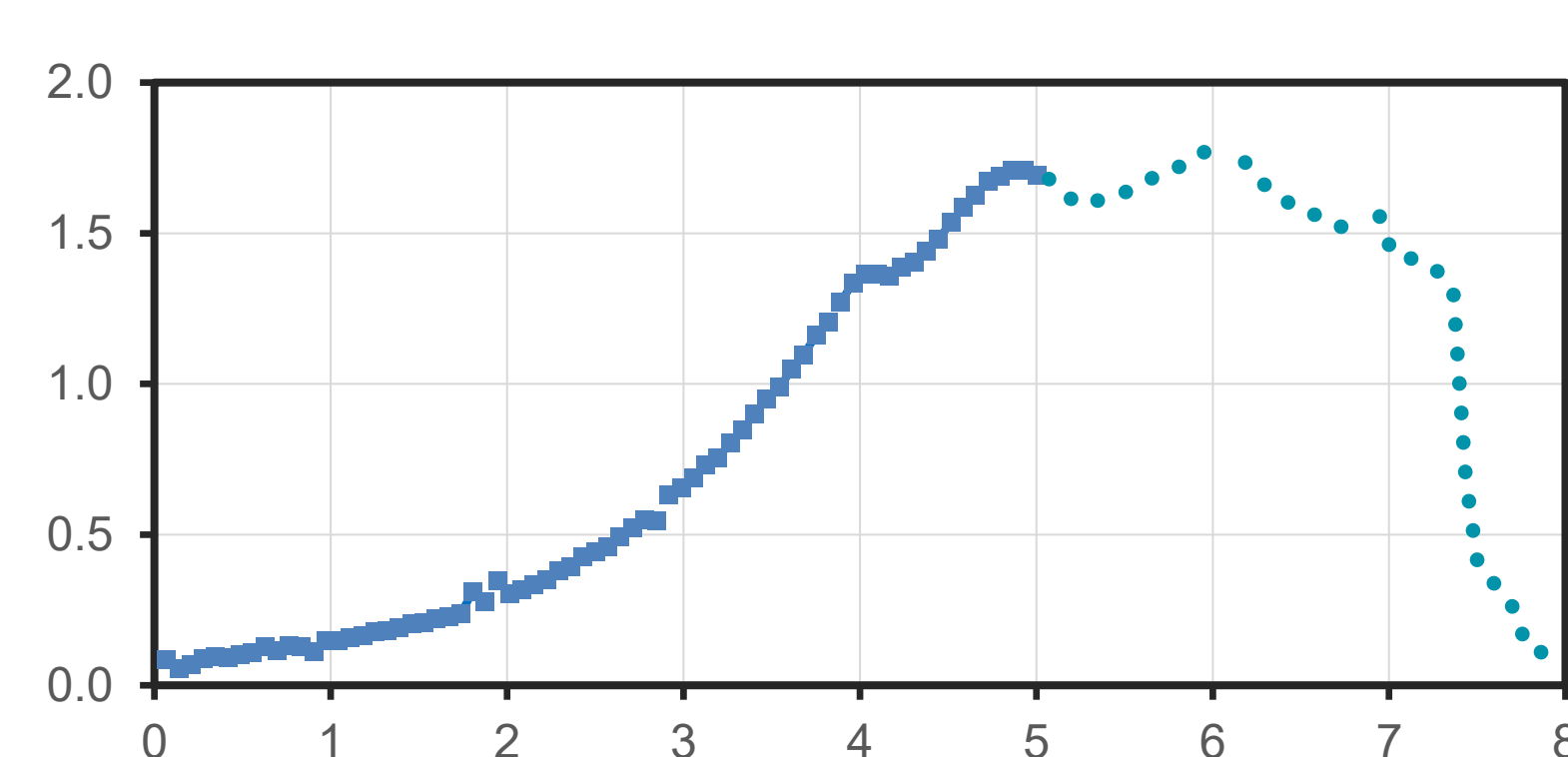


Figure 4: Online-data CHO DP12, TC42 medium

Online prediction of VCD, Glucose and Lactate

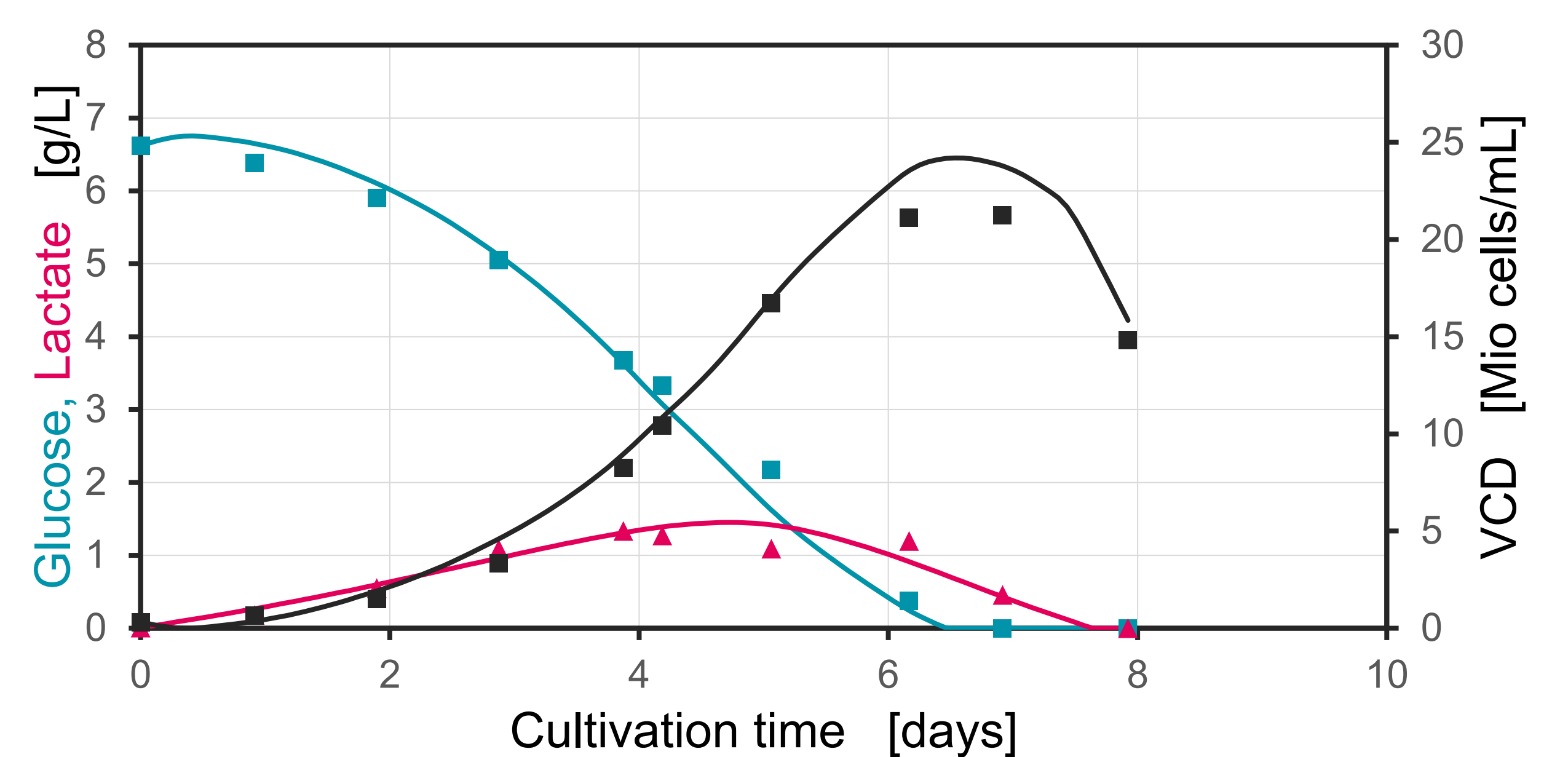


Figure 5: Online-predicted VCD, Glucose and Lactate concentration for CHO DP12, TC-42 medium, based on hybrid model

Conclusion

- ✓ Oxygen consumption and carbon dioxide production are well connected to VCD and metabolite concentration
- ✓ Hybrid modelling can predict VCD, Glucose and Lactate online
- ✓ Continuous data without sampling and delayed analysis

- [1] Neuss et al. (2024) New Biotechnology, <https://doi.org/10.1016/j.nbt.2024.09.008>
 [2] Neuss et al. (2024) Biotechnology Progress <https://doi.org/10.1002/btpr.3468>
 [3] Ihling et al. (2022) Biotechnol. J., <https://doi.org/10.1002/biot.202100677>
 [4] Reyes et al. (2025) J. of Biotech., <https://doi.org/10.1016/j.jbiotec.2025.01.008>

Kuhner Shaker
Dinkelbergstr. 1
CH-4127 Birsfelden
onlinetechnology@kuhner.com
www.kuhner.com

We gratefully acknowledge the support of our project partners from University of Barcelona, University of Osaka, Technical University of Hamburg, Technical University of Munich and RWTH Aachen University for providing training datasets to this project.

