

# Novel 96 well screening tool for time-resolved, independent and non-invasive determination of the Oxygen transfer rate

Juan C. Porras<sup>1\*</sup>, Andreas Schulte<sup>1</sup>, Lara Pauline Munkler<sup>2</sup>, Peter Becker<sup>2</sup>

<sup>1</sup>Office Germany, Kuhner Shaker GmbH., Herzogenrath, North Rhine Westphalia, Germany \* presenting author  
<sup>2</sup>Enduro Genetics ApS, Ole Maaløes Vej 3, 2200 Copenhagen, Denmark

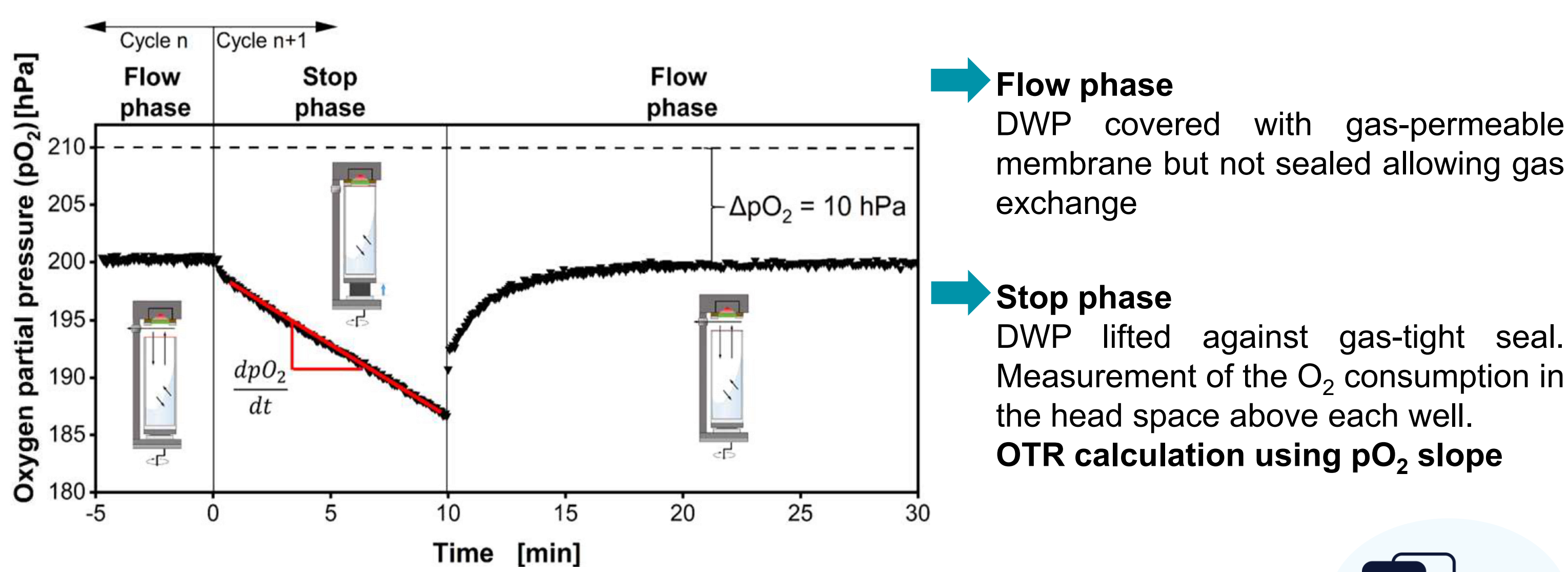
## Introduction

Conventional mini-bioreactor systems with integrated online-monitoring lack the technology to measure the essential process parameter **Oxygen Transfer Rate (OTR)**. This crucial parameter can be used to distinguish between typical biological phenomena such as substrate limitation, oxygen limitation, or diauxic growth. The novel **Kuhner microTOM** is an **online-monitoring tool for non-invasive, high-throughput determination of the OTR in deep-well microtiter plates (DWP)**. A reported coefficient of variation < 2% for OTR determination indicates good reproducibility of parallelized respiration measurements in microbial and cell culture application, supporting the reliability of the device for comparative screening studies [1-2]. Enduro Genetics ApS is using the OTR delivered by Kuhner microTOM to establish and tech transfer predictive small-scale screening when implementing titer-increasing genetic biosensor technology into industrial production strains of *E. coli*, *B. subtilis* and yeasts.



## Kuhner microTOM function

The microTOM system operates as a multi-channel platform with 96 positions enabling **independent determination of the OTR of all wells** in a standard 96 DWP. The measurement uses optical sensor spots based on dynamic fluorescence quenching, allowing contactless detection of oxygen partial pressure ( $pO_2$ ) in the head-space without consumption of  $O_2$  or interference with the biological system [1]. The *microTOM Control* software automatically realizes OTR readings during the whole cultivation as described below.



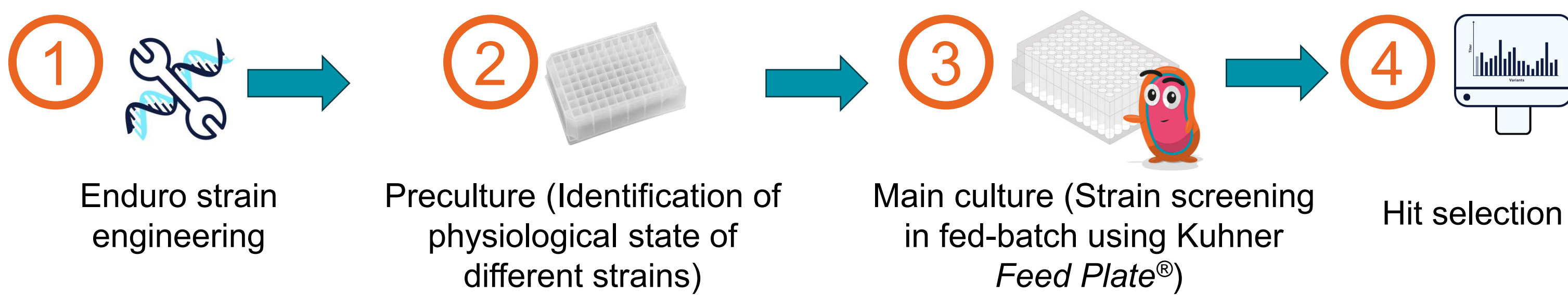
- Flow phase**  
DWP covered with gas-permeable membrane but not sealed allowing gas exchange
- Stop phase**  
DWP lifted against gas-tight seal. Measurement of the  $O_2$  consumption in the head space above each well. **OTR calculation using  $pO_2$  slope**

## 1 Background Enduro

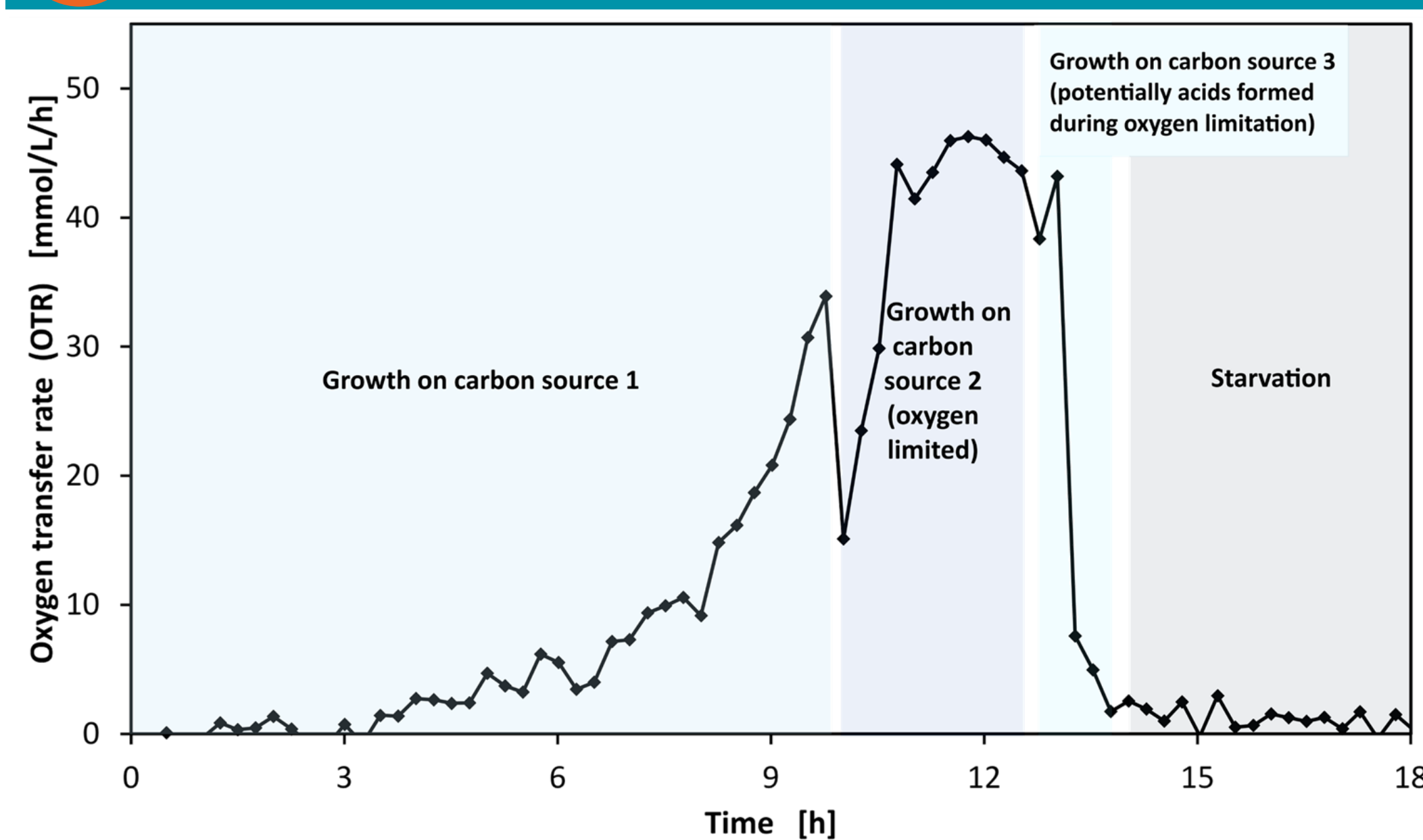
To overcome productivity decline in industrial bioprocesses, Enduro developed its *EnduroSense*<sup>®</sup> platform, which genetically links cell growth to high product formation using genetic biosensor-empowered plug-ins. This product-agnostic technology selectively enriches high-producing cells and is broadly applicable across products and industrial microorganisms, including bacteria and yeasts [3-5]. To successfully translate strain improvements from DWP-based screening protocols into industrial bioprocesses, Enduro employs OTR monitoring using the Kuhner microTOM system to significantly improve plate-to-tank predictability when implementing *EnduroSense*<sup>®</sup> in client production strains.

## OTR-guided DWP screening protocol development

**Goal:** Development of a highly predictive small-scale screening protocol



## 2 Diauxic growth of E. coli on different carbon sources

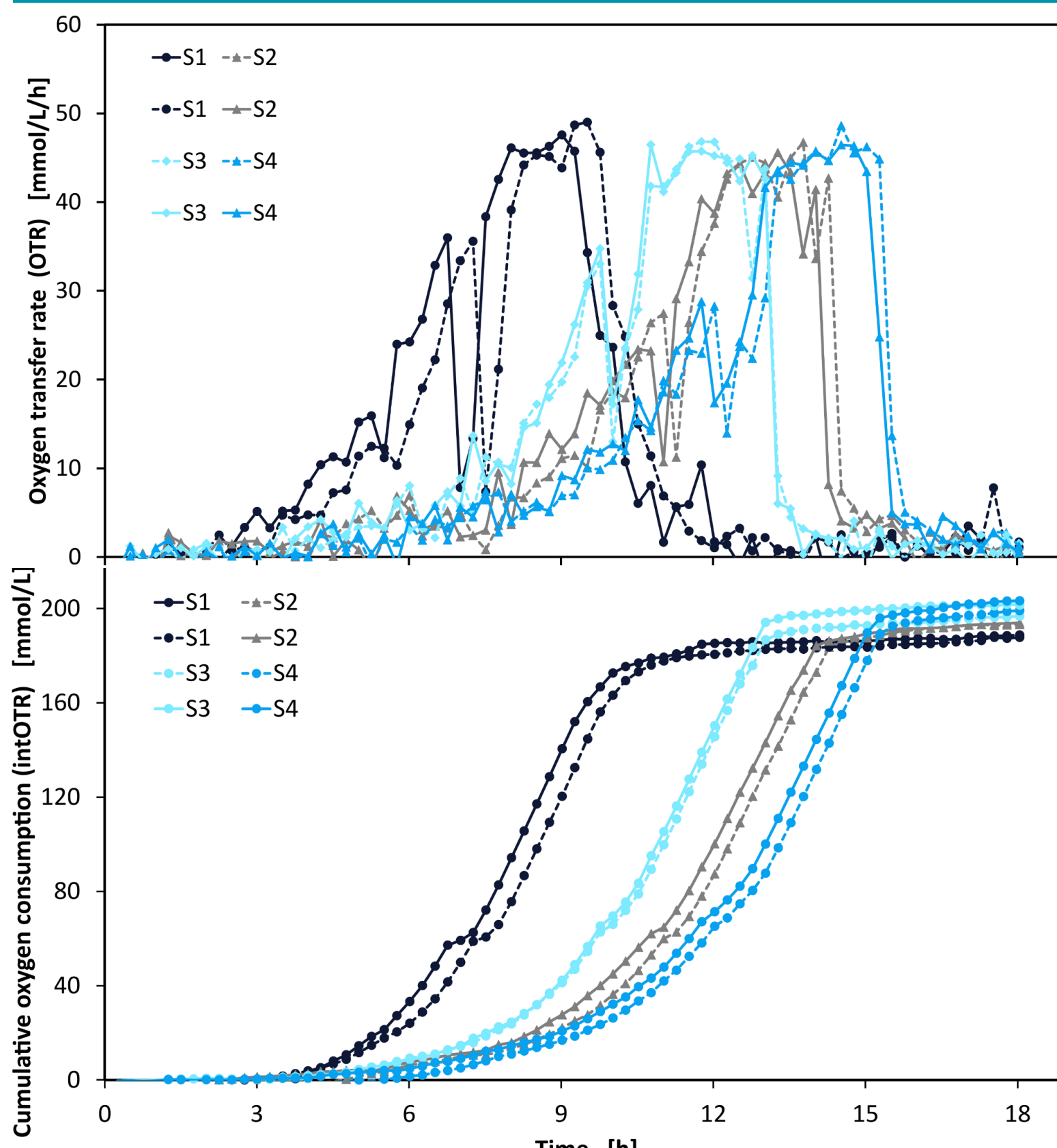


Diauxic growth of *E. coli* on different carbon sources

Preculture medium with 2 different carbon sources inoculated with 1 colony in 96 v-bottom square DWP

- Precise insights into physiological state of the culture during growth on a preculture medium with two different major carbon sources
- Diauxic shift between the two major carbon sources is clearly visible
- Even the re-assimilation of overflow metabolites can be detected

## 2 Precise monitoring of pre-cultures



**Preculture** growth of 4 different *E. coli* strains in semi-defined medium with 2 carbon sources in 96 v-bottom square DWP (n=2)

**Online monitoring of OTR in the Kuhner microTOM allows to:**

- Determine specific growth phases of 4 *E. coli* strains in minimal medium with 2 carbon sources
- Identify optimal transfer timing to main culture

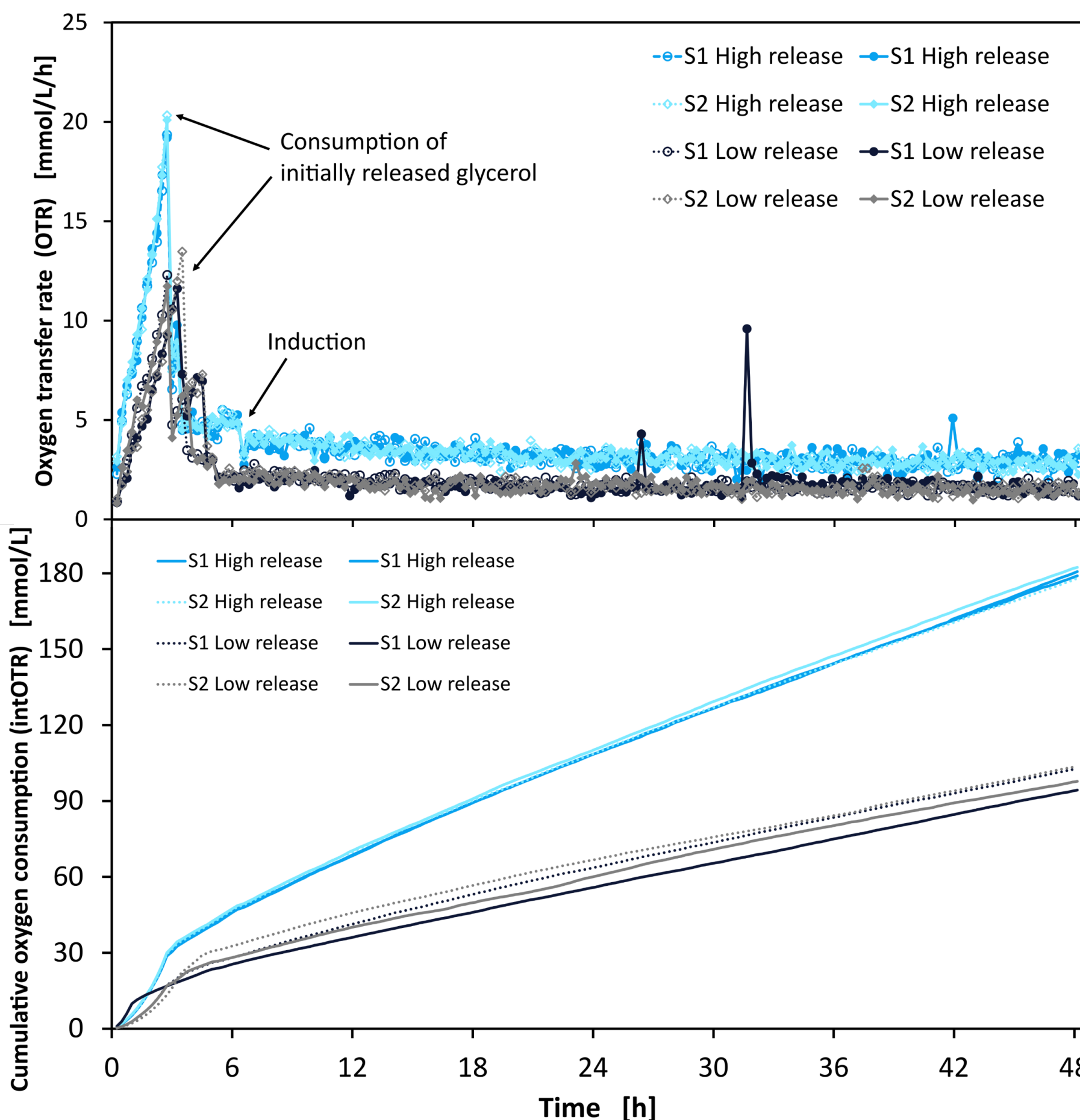
## 3 OTR-guided induction in fed-batch DWP

Feeding strategy: Fed-batch in DWP using Kuhner *Feed Plate*<sup>®</sup> for scale-up relevant strain screening process

**Method A:** Conventional induction using **offline OD<sub>600</sub>** measurements

- Many interruptions during process
- Labor-intensive
- No insights into physiological state of the culture
- Volume changes impact mixing

**Method B:** OTR-guided induction using the **Kuhner microTOM**



- Online monitoring of the OTR without process interruptions
- Identification of ideal induction time point (all strains are carbon-limited)
- Confirmation of selection of correct initial carbon source concentration to avoid oxygen limitation
- Additional: Calculation of growth rate based on high resolution online signal

## Conclusion

Enduro employs the Kuhner microTOM technology offering the following benefits:

- Truly **non-invasive online-monitoring of OTR**
- Data-driven bioprocess development** at high-throughput with sufficient replicates for data robustness
- Deeper understanding** of complex bioprocess cultivations enables improved plate-to-tank predictability
- Compatible with Kuhner *Feed Plate*<sup>®</sup> technology enabling to implement **fed-batch strategy in DWP**

References

- [1] Dinger et al. (2021) *Biotechnology Bioengineering*, <https://doi.org/10.1002/bit.28022>.
- [2] Neuss et al. (2024) *Biotechnology Progress*, <https://doi.org/10.1002/btpr.3468>.
- [3] Rugbjerg et al. (2018) *Proceedings of the National Academy of Sciences*, 115(10), 2347–2352.
- [4] Rugbjerg et al. (2018) *Nature Communications*, 9, 787.
- [5] Rugbjerg et al. (2019) *Nature Biotechnology*, 37(8), 869–876.

