

Fed-Batch Micro Plates

INTRODUCTION

Screening

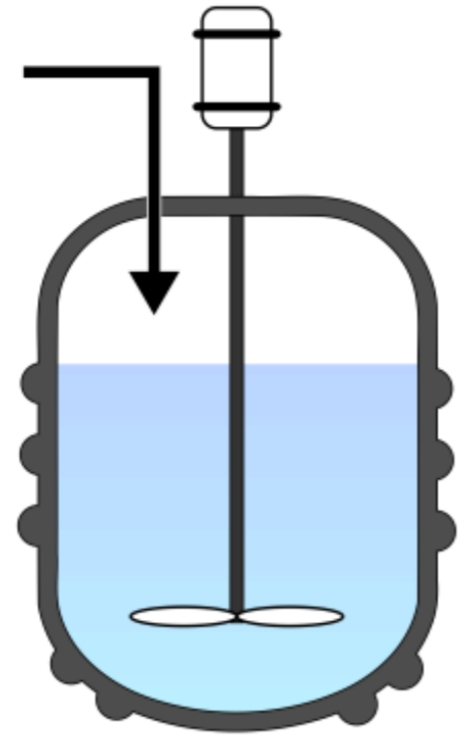


Batch
operation
mode



Selection of
suboptimal
production strain!

Production



Fed-Batch
operation
mode

- Varying operation modes result in different physiological conditions and performance of strains due to:

- Catabolite repression
- Overflow-metabolism
- Substrate inhibition
- Osmotic pressure

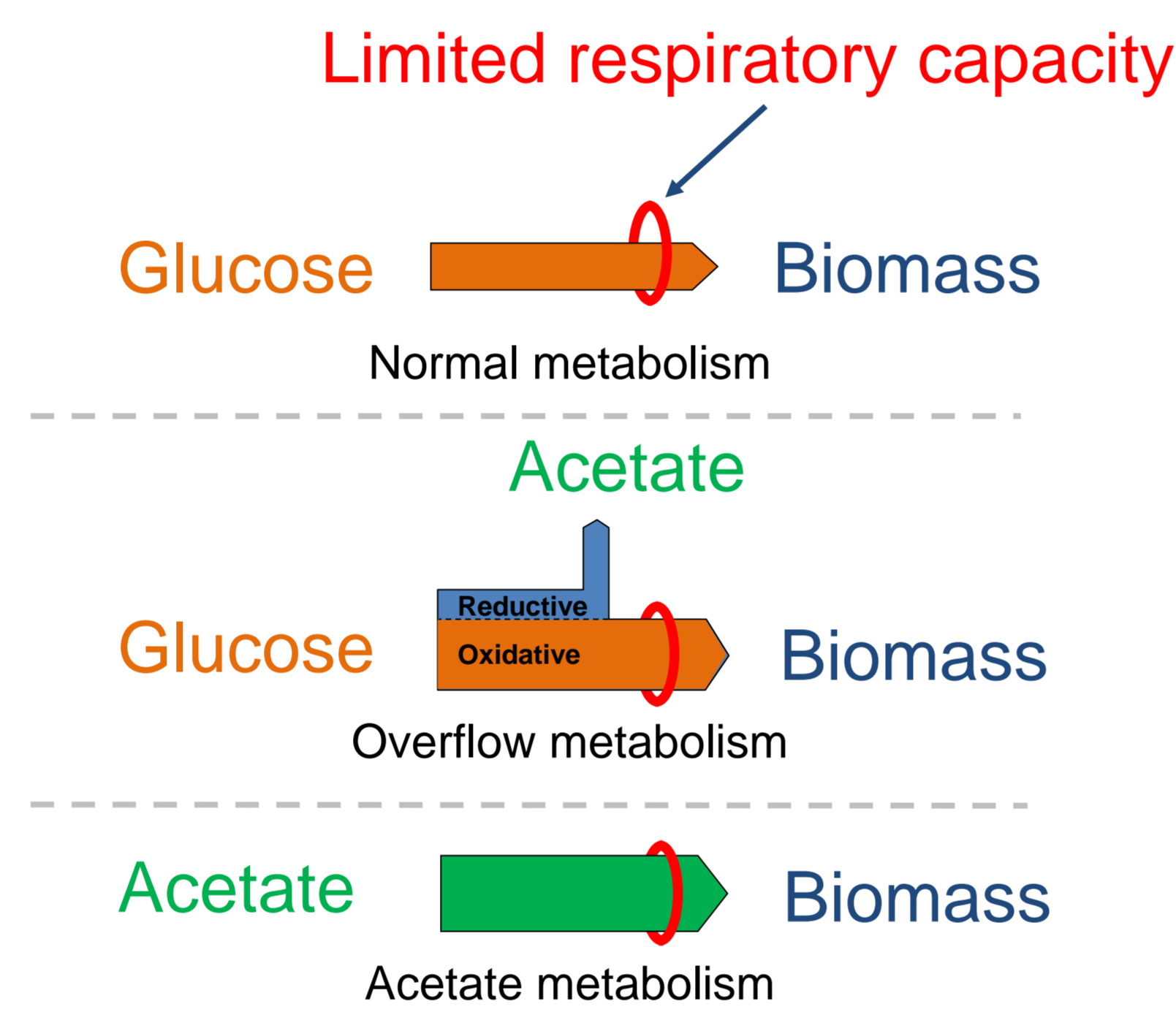
- **Demand: Fed-Batch micro plate**

DEVELOPMENT OF THE RELEASING SYSTEM

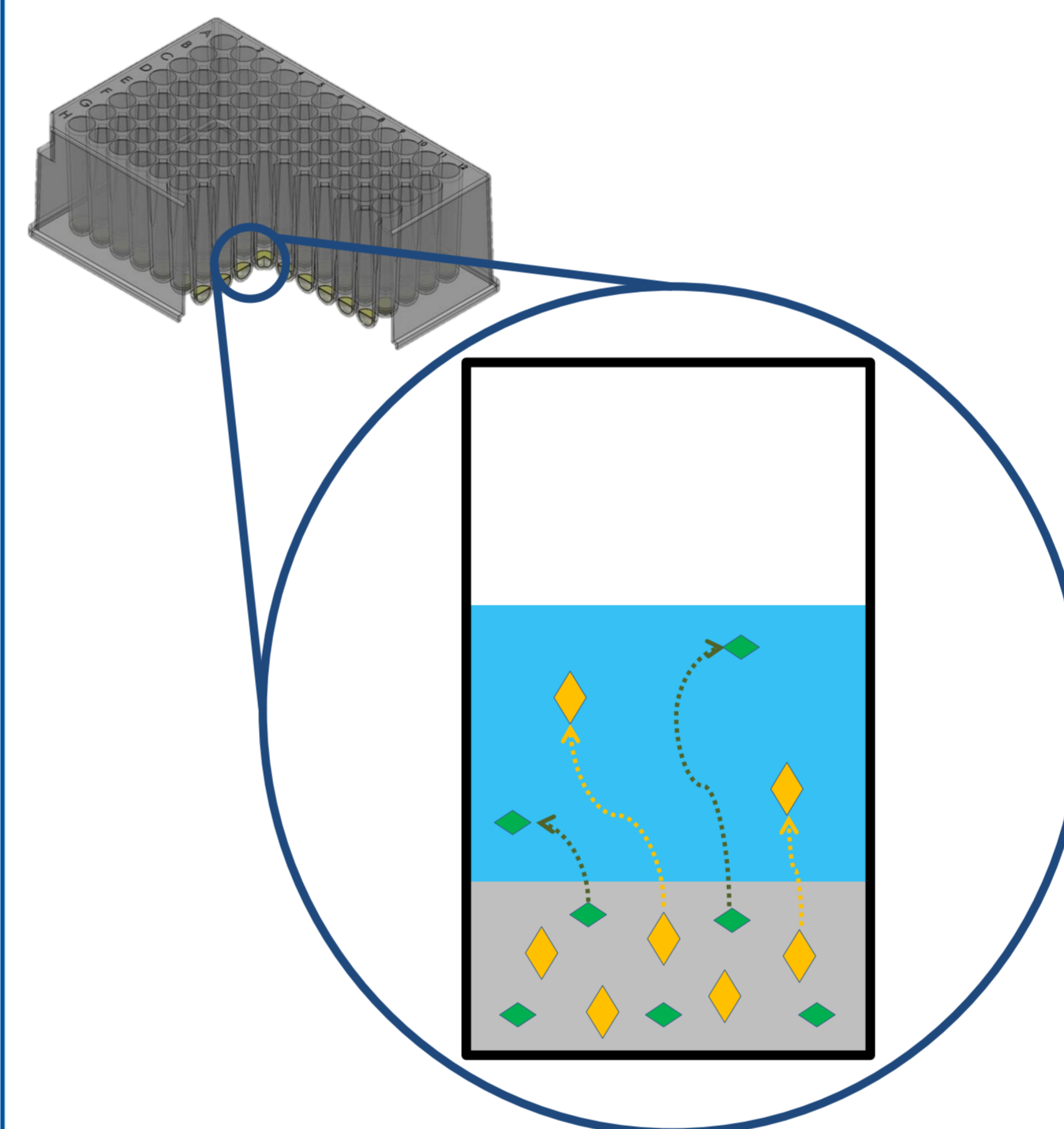
1) Modelling (of *E.coli*)

Aim:
Reduce experimental effort for the:

- Determination of nutrient demand
- Description and quantification of necessary pH stabilization agent

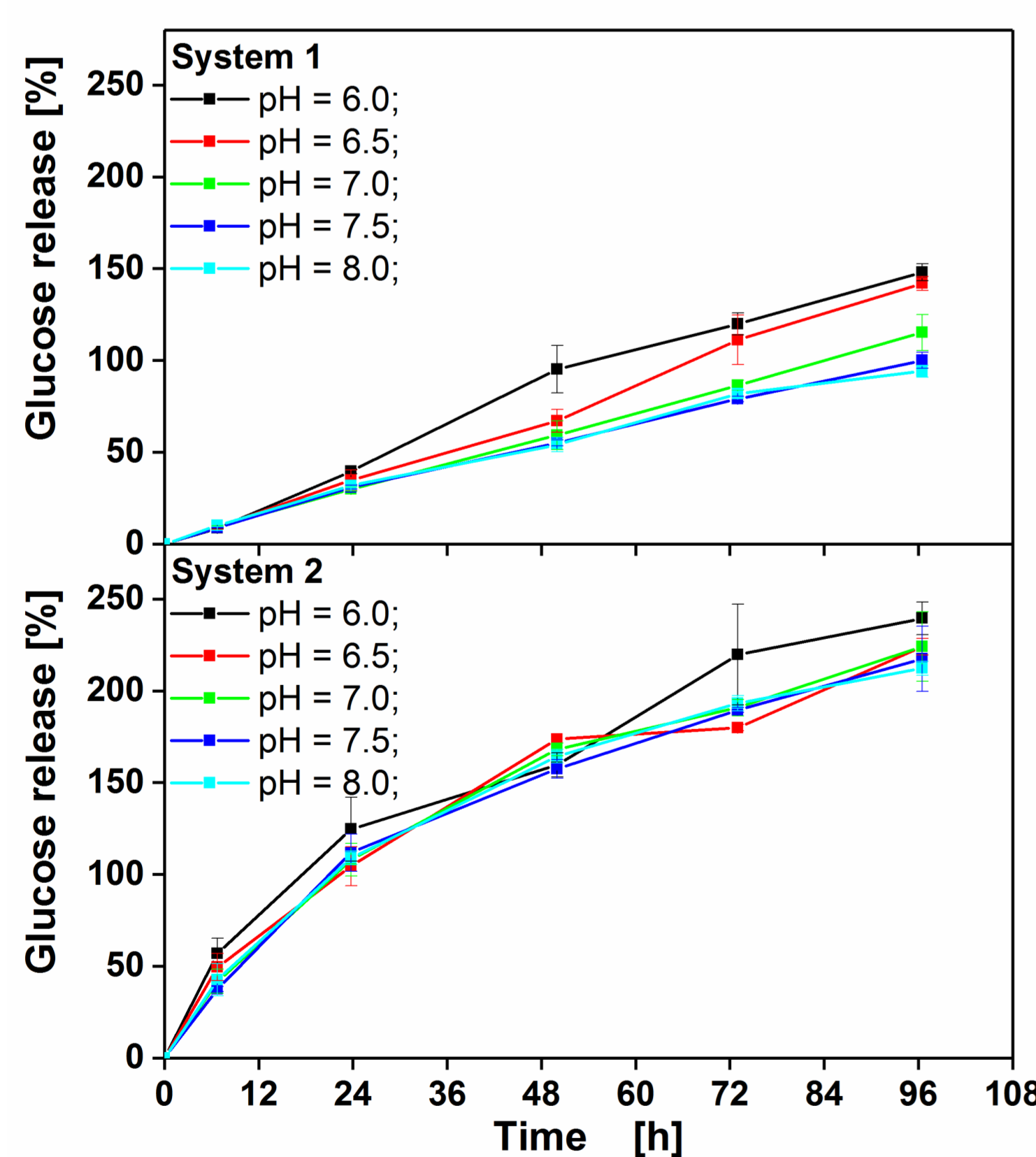


2) Technical development



- Polymeric-based feeding system (two systems)
- Siloxane matrix at the bottom of each well
- Substrate (♦) embedded, optionally with pH stabilization agent (♦)
- Once in contact with medium → defined release over time

PRIMARY RESULTS



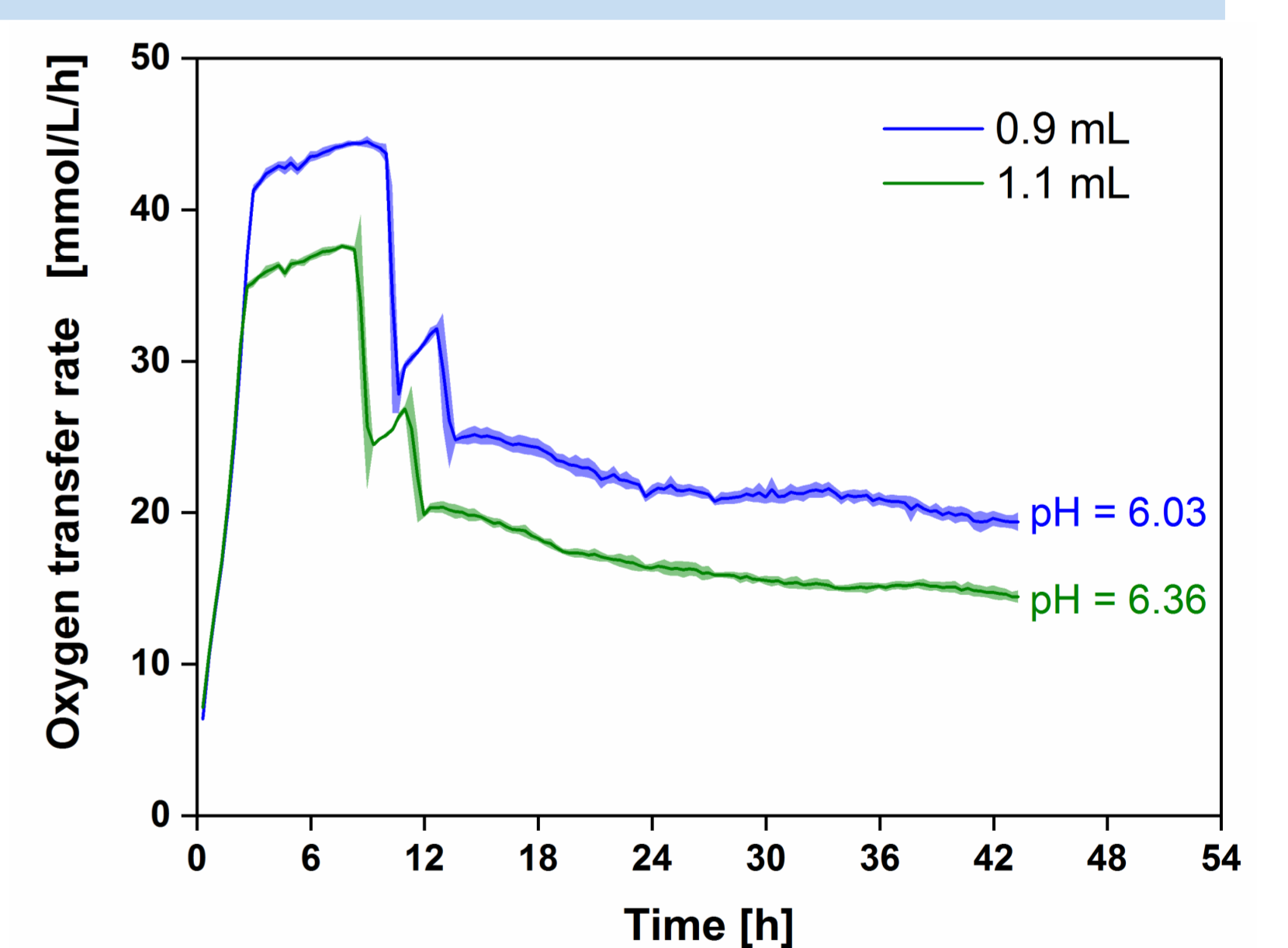
◀ **Release kinetics of glucose from Fed-Batch micro plate systems in media of different pH.** 100 % Reference: System 1, 96 h, pH 7.5. No biology was applied. Wilms medium adapted from Jeude et al. (2007)^[1]. MOPS buffer, 96-deep-well square micro plate, T = 37°C, V_L = 1000 μL/well, osmolality = 0.6 osmol/L, 350 rpm, d₀ = 50 mm.

- System 1: Glucose release depends on pH, linear kinetics
- System 2: pH shows less impact on glucose release, declining kinetics

→ Different feed plates for many kinds of applications

▶ ***E.coli* BL21 DE3 cultivation in 48 round Fed-Batch glucose micro plate, system 2.** Wilms medium, MOPS buffer 200 mM, pH₀ = 7.5, T = 37°C, n = 1000 rpm, d₀ = 3 mm, Inoculum: OD = 1, six replicates, Oxygen transfer rate measurement in μRAMOS-device from Flitsch et al. (2016)^[2].

- Different feeding rates due to varying filling volumes
- Opportunity for Fed-Batch cultivation with oxygen transfer rates up to 25 mmol/L/h



→ Varying feeding strategies for process optimization and screening procedures possible

CONCLUSION AND OUTLOOK

- Fed-Batch cultivation in micro plate with two different releasing systems successful
- In future tailor-made Fed-Batch micro plates will be developed for each organism supported by model
- Validation of Fed-Batch plate by conducting screening experiments with subsequent scale-up



Fed-Batch

Selection of **optimal**
production strain!

Fed-Batch