

Integration of a robotic small scale bioreactor system as a prerequisite for a self-learning & autonomous cultivation platform

Lucas Kaspersetz^{1,2}, Saskia Waldburger¹, Marie-Therese Schermeyer^{1,2}, Sebastian Hans^{1,2}, Mariano Nicolas Cruz Bournazou^{1,2,3}, Peter Neubauer^{1,2}



¹Technische Universität Berlin, Chair of Bioprocess Engineering, Straße des 17. Juni 135, 10623 Berlin, Germany

²Technische Universität Berlin, KIWI-biolab, Straße des 17. Juni 135, 10623 Berlin, Germany

³DataHow AG, Vladimir-Prelog-Weg 1, 8093 Zurich, Switzerland



www.kiwi-biolab.de

www.tu.berlin/bioprocess

Motivation / Introduction

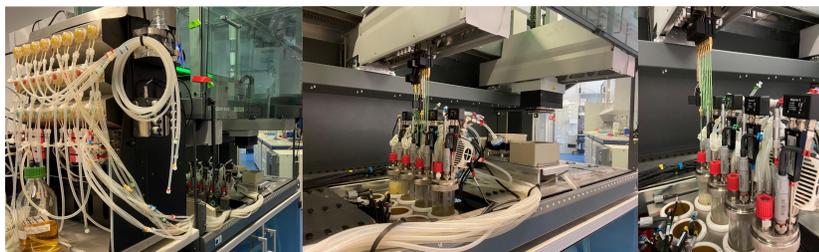
The process industries are moving towards digitalization and automation, known as the industry 4.0. On the contrary, the biotechnological sector lacks this transition and does not even fulfill the automation standards of industry 3.0, while facing the necessity of faster development cycles [1]. In order to enhance automated high-throughput process development (HTBD) and optimization under consistent scalable conditions, we present the integration of a commercially available small scale bioreactor system, a liquid handling system (LHS) & a microplate reader into our HTBD-robotic facility [2]. The integration and data management follows the F.A.I.R data principle [3], storing the corresponding on-line, at-line & off-line measurements of each bioreactor, as well as the executed liquid handling steps, in a centralized database, enabling further deployment. An initial fed-batch cultivation with automated sampling is used for verification of the conceptual design of the facility.

Concept and Results

BioXplorer Facility

8x parallel cultivation Automated sample handling At-line analysis

- Cultivation system HEL BioXplorer 100
- LHS Tecan Evo 150
- Microplate reader



Cultivation system

- 8 vessels à 25-150 mL, 1 MFC per vessel, 3 peristaltic pumps per vessel, batch, fed-batch & continuous mode

Monitoring on-line

- pH, DO, turbidity, temperature, stirring speed, feed rate, air flow rate,

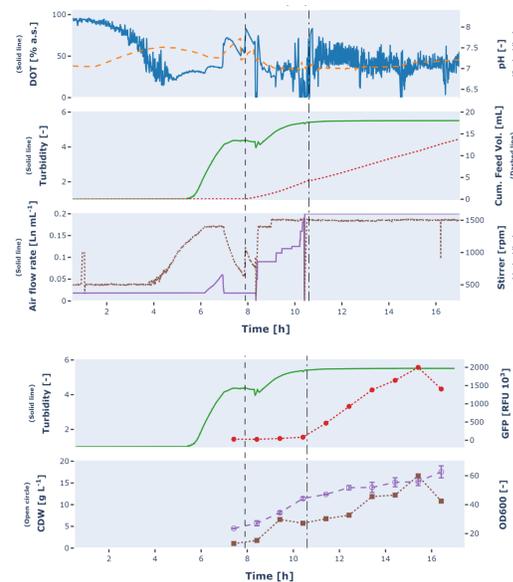
Monitoring at-line

- automated sampling for substrate, metabolites, product, biomass,

Individual process control

- pH, DO, temperature, stirring speed, air flow rate, pump rates, inducer addition,

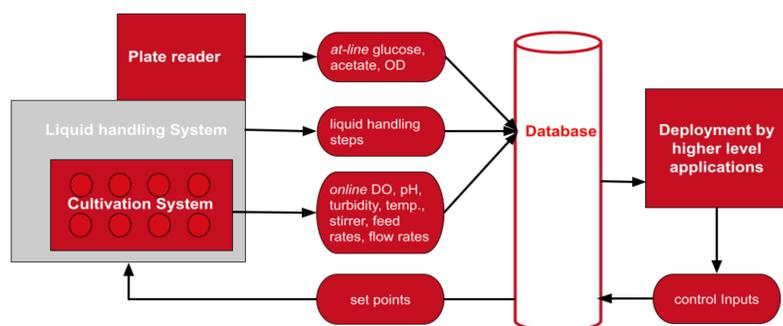
Proof of Concept



Figures Upper: *Online* process data for DOT, pH, turbidity, cumulated feed volume, air flow rate & stirrer speed. Lower: *Online*, & *offline* data for biomass & *at-line* data for fluorescence of GFP. First vertical dashed line indicates feed start & second vertical dashed/dotted line indicates induction.

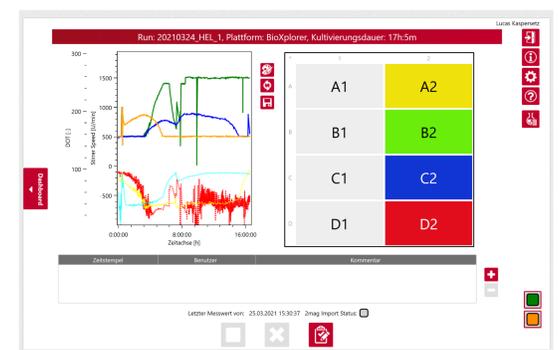
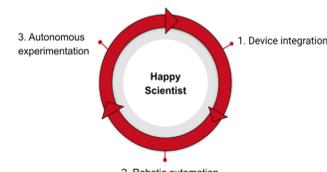
E. coli BL21 (DE3) with GFP-fusion (pET28 based reporter system) as protein of interest cultivated in fed-batch process (batch, exponential phase followed by induction phase). *Online* process data was logged every 30s, while *at-line* samples for OD₆₀₀ and fluorescence's (479 nm excitation, 520 nm emission) and *off-line* samples for CDW were taken every hour. *Online* turbidity measurements were limited at higher cell densities > 12 g L⁻¹ and complemented through *at-line* OD₆₀₀ and *offline* CDW measurements. Additionally, fluorescence measurements allow for product quantification, Hence, valuable process insights can be gained.

Integration into HTBD facility



Integration into centralized database

- *on-line* & *at-line* data submission of all devices
- reading external set points
- prerequisite for further deployment/ automation steps



Live data in centralized database allows for further automation steps, data driven process development and deployment by modelling frameworks and artificial intelligence based tools developed in the KIWI-biolab.

Conclusions and Outlook

- I. Successful integration of H.E.L BioXplorer into liquid handling station for automated sampling procedure
- II. Conceptual design validated by an industrial relevant fed-batch cultivation
- III. Additional process insights through *at-line* and *off-line* process data, which enable data driven real-time optimization
- IV. Connection to centralized database as a prerequisite for further automation steps and deployment by AI based tools
- V. Finalize integration steps for remote set point writing, and logging of LHS steps
- VI. Further automate sampling and metabolite analysis by integration of a mobile lab assistant into the HTBD facility

Acknowledgements / References

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