Multi-cycle high-throughput growth media optimization using batch Bayesian optimization

Frédéric M. Lapierre[1,2], Pasquale Mattaliano[2], Dennis Raith[2,3], Mariela Castillo-Cota[2,3], Jonas Bermeitinger[2], Neda Sabet[2], and Robert Huber[1]

Abstract

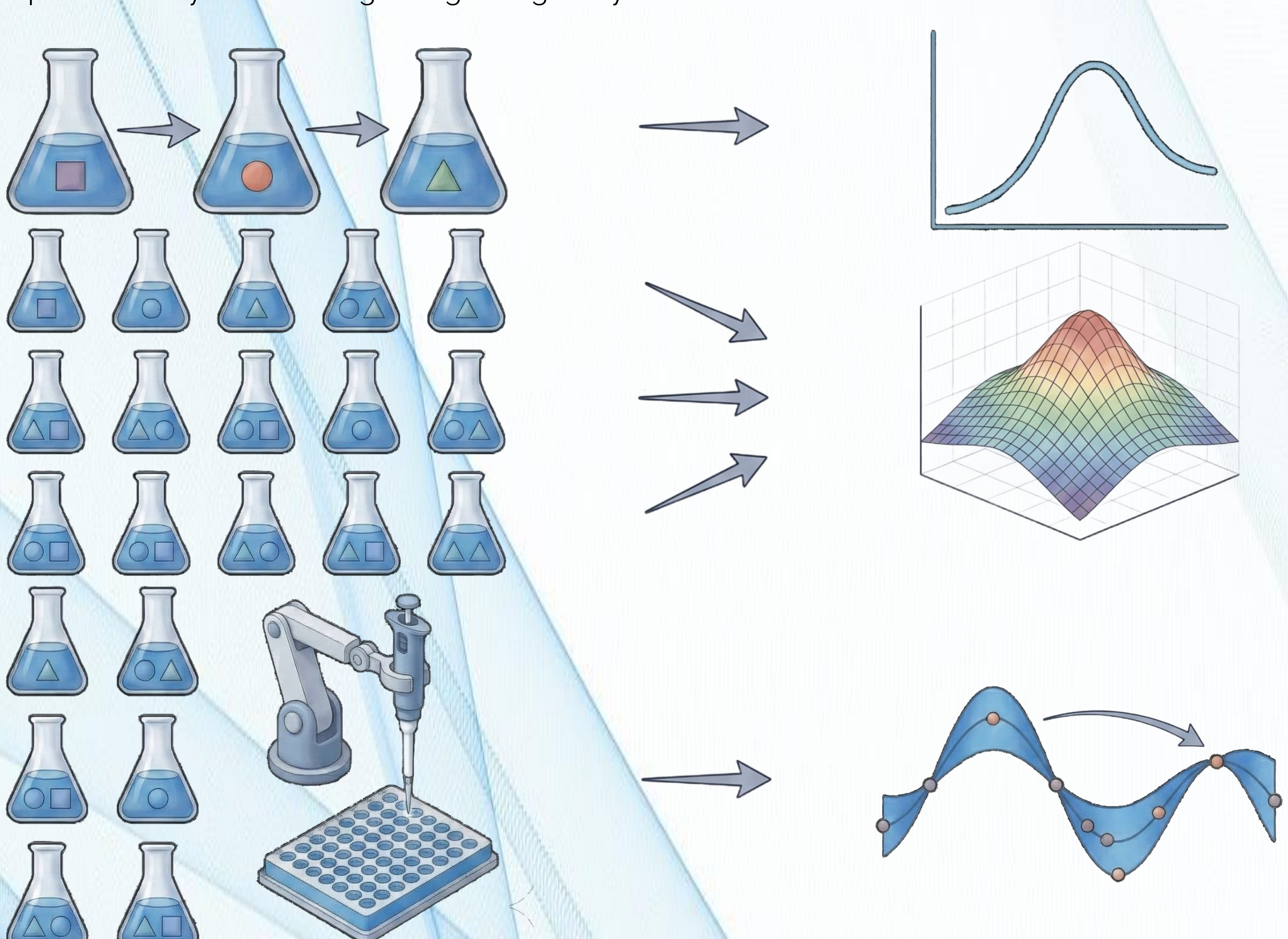
The **optimization of growth media** is a crucial step in enhancing microbial growth and product formation in industrial bioprocesses. While **Design of Experiments (DoE)** is widely used for this purpose, it has **inherent limitations**, particularly in **capturing complex interactions** between media components and managing the high number of required experiments without overlooking important factors or interactions. This study evaluates **batch Bayesian optimization (BBO) as an alternative** approach by applying it in **simulations, in wet-lab experiments, and bioreactor**, using Sporosarcina pasteurii as a model organism, to demonstrate its effectiveness.

In silico, **BBO significantly outperformed DoE** on average (+14%), although it was not superior in all test cases, highlighting the need for further investigation. In automated high-throughput microbioreactor experiments with S. pasteurii, BBO led to a **28% increase** in maximum backscatter value, indicating a higher biomass titer compared to a DoE-optimized medium. BBO enabled a more targeted optimization of media components, allowing broader exploration when needed and focusing on specific adjustments where appropriate. The BBO-optimized growth medium, designed in small-scale experiments, also outperformed the DoE-optimized medium at a 2 L bioreactor scale by 19%.

In our use cases, BBO offers a **more effective and targeted approach** for growth media optimization than traditional DoE. Placing our results in the context of other studies, BBO shows promise for outperforming DoE in bioprocess optimization tasks. The ability of BBO to streamline the optimization process could **enhance yields and reduce costs** in industrial bioprocesses.

Introduction: Navigating Optimization Methods

Industrial fermentation success hinges on cost-effective, strain-specific culture media. Traditional **one-factor-at-a-time (OFAT)** methods are inefficient and miss complex interactions, while **Design of Experiments (DoE)** often struggles with the nonlinear nature of microbial growth. Bayesian Optimization (BO) offers a superior alternative by using Gaussian process surrogate models to balance exploration and exploitation. Specifically, **Batch Bayesian Optimization (BBO)** enables the simultaneous identification of multiple sampling points, drastically reducing the time required for optimization cycles in slow-growing biological systems.



From top to bottom one-factor-at-a-time, Design of Experiments and automated Batch Bayesian Optimization approaches.

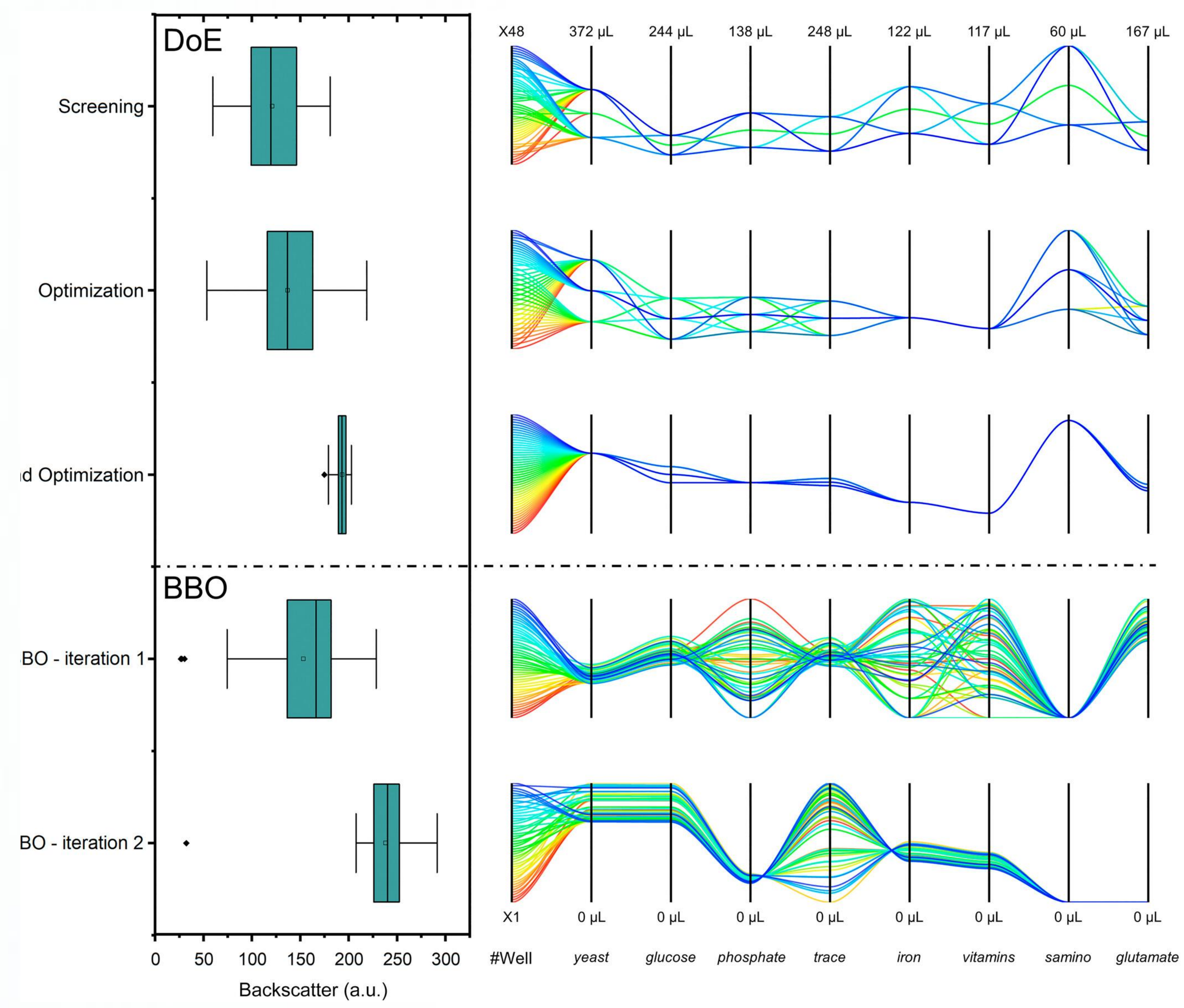
Methodology: The BBO Framework & Automated Hardware

The methodology employed in this study combines advanced **probabilistic modeling** with **high-throughput automation** to optimize the growth of Sporosarcina pasteurii. The BBO process begins by constructing a **Gaussian process (GP) model** from an initial data set, which provides both **function estimates** and **uncertainty measurements** critical for navigating the search space. To manage the computational complexity of selecting multiple points for a batch, the system adopts a heuristic strategy using local penalization to define exclusion zones around selected points, ensuring diverse exploration without redundant evaluations.

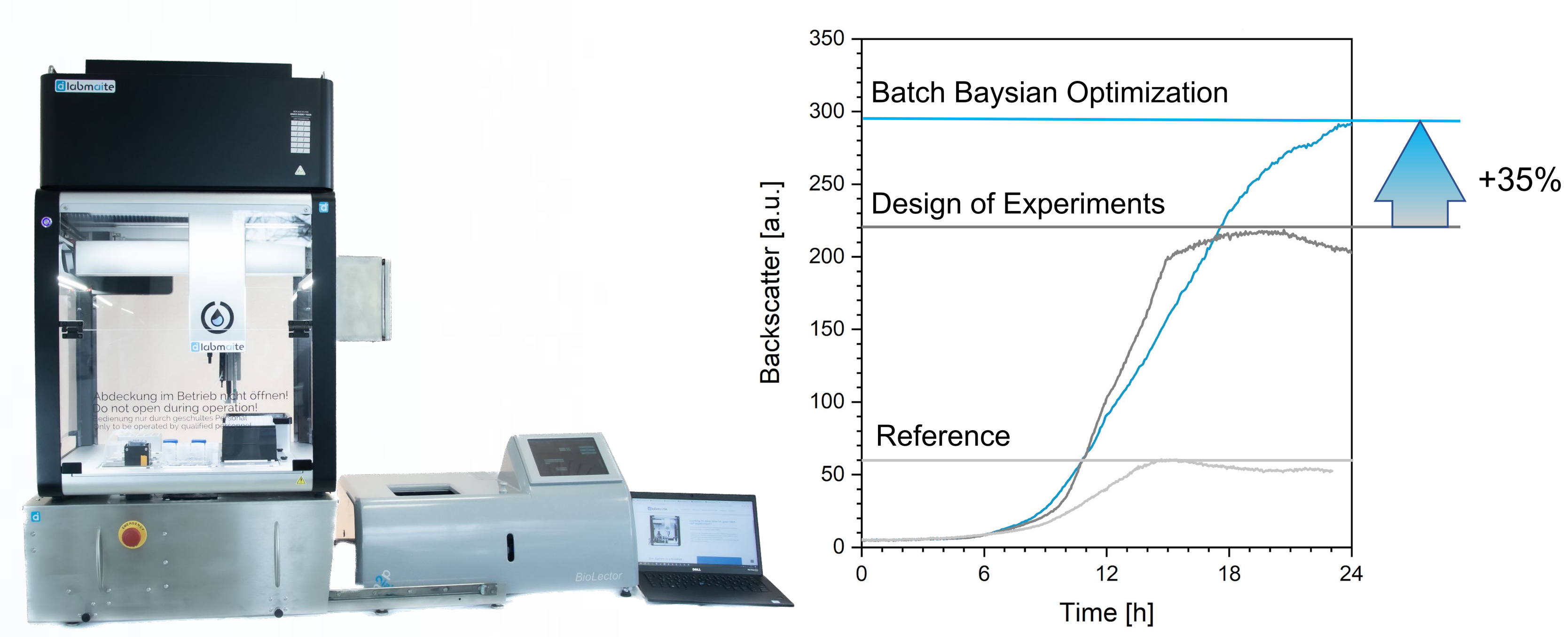
This study optimized the growth of **Sporosarcina pasteurii** by integrating **probabilistic modeling** with high-throughput automation. The BBO framework utilizes Gaussian processes to map the search space and local penalization heuristics to ensure **diverse**, **non-redundant** batch selection. This algorithmic framework is integrated with a **fully automated microbioreactor setup** consisting of a BioLector I system for **parallel cultivation** and **real-time monitoring** of biomass via backscatter. A **liquid handling system** (OT-2) is utilized to dispense media components directly into the microplates, while a small-scale turbidostat system ensures the continuous provision of a consistent inoculum for multiple automated cycles.

Results: Superior Exploration and Scalability

BBO outperformed DoE by maintaining a broader exploration of the design space. While DoE dismissed iron early as insignificant, BBO's iterative approach identified it as a critical limiting factor at high biomass concentrations. Consequently, the BBO-optimized medium achieved a **14% increase *in-silico* simulation**, **28% increase** in maximum backscatter in **microscale trials.** In a 2L **laboratory bioreactor**, BBO demonstrated excellent scalability, yielding an OD600 of 67.4, a **19% improvement** over DoE and the highest batch cultivation density for *S. pasteurii* reported in literature to date.



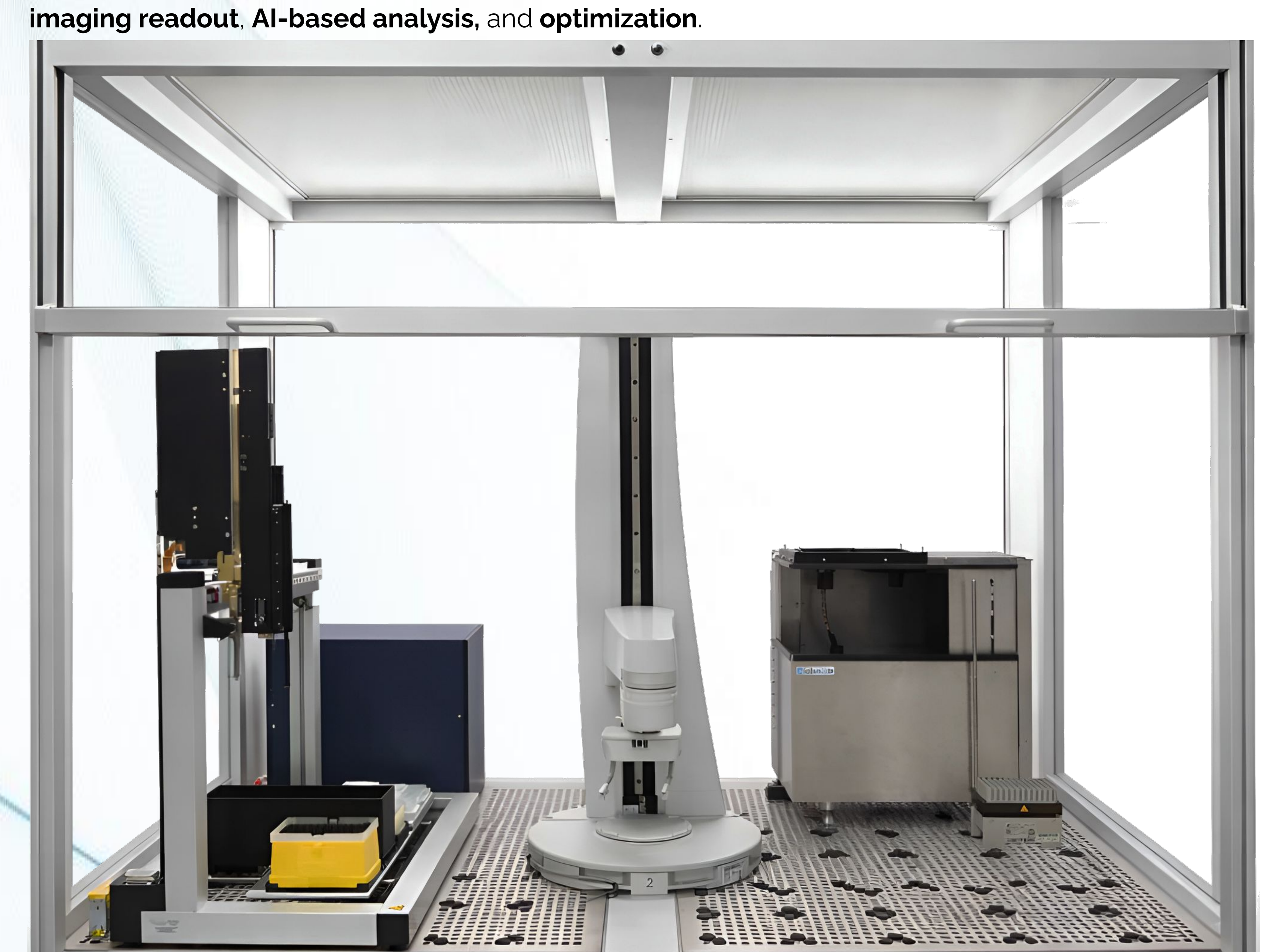
DoE vs. BBO variable exploration and optimization results



Automatic cultivation device and growth data for the microbioreactor cultivation of bacterium *Sporosarcina pasteurii*.

Outlook

Future investigations should **expand** the application of BBO to optimize actual **target product concentrations** and **fed-batch process parameters** like feed composition and rates. Additionally, the effectiveness of BBO should be further assessed against other advanced optimization algorithms and applied to a **broader range of objectives**, such as **optimizing product yield per media cost**. Another further investigation following this study is **integration of BBO with high-throughput automation** which marks a significant transition toward fully autonomous, **set-and-forget intelligent systems** in bioprocess development. This **closed-loop learning system** can **automate the entire experimental lifecycle;** from **high-precision liquid handling** and **media formulation** to **real-time imaging readout**, **AI-based analysis,** and **optimization**.



High-throughput self optimizing system including liquid and plate handler, microscope, pipetting robot and incubator.

Scan to learn more

1. Department of Engineering and Management, HM Hochschule München University of Applied Sciences, Munich, Germany
2. Labmaite GmbH, Freiburg im Breisgau, Germany
3. Department of Computer Science, University of Freiburg, Freiburg im Breisgau, Germany

Bundesministerium für Bildung und Forschung

info@labmaite.com
labmaite.com
Türkheimerstraße 4a, 79110 Freiburg, Germany