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

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Abstract

Optimizing microbial media is vital for industrial yields, yet traditional **Design of Experiments (DoE)** often **misses complex interactions**. This study evaluates **Batch Bayesian Optimization (BBO)** using ***Sporosarcina pasteurii.***

In-silico, **BBO outperformed DoE** by **14%**. In automated **wet-lab trials**, BBO achieved a **28%** increase in biomass titer via **superior exploration of media components.** These results scaled successfully to a **2L bioreactor**, where BBO yielded **19%** more biomass than DoE. Overall, BBO provides a **more efficient, targeted** framework for **enhancing bioprocess yields** and **reducing costs.**

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.

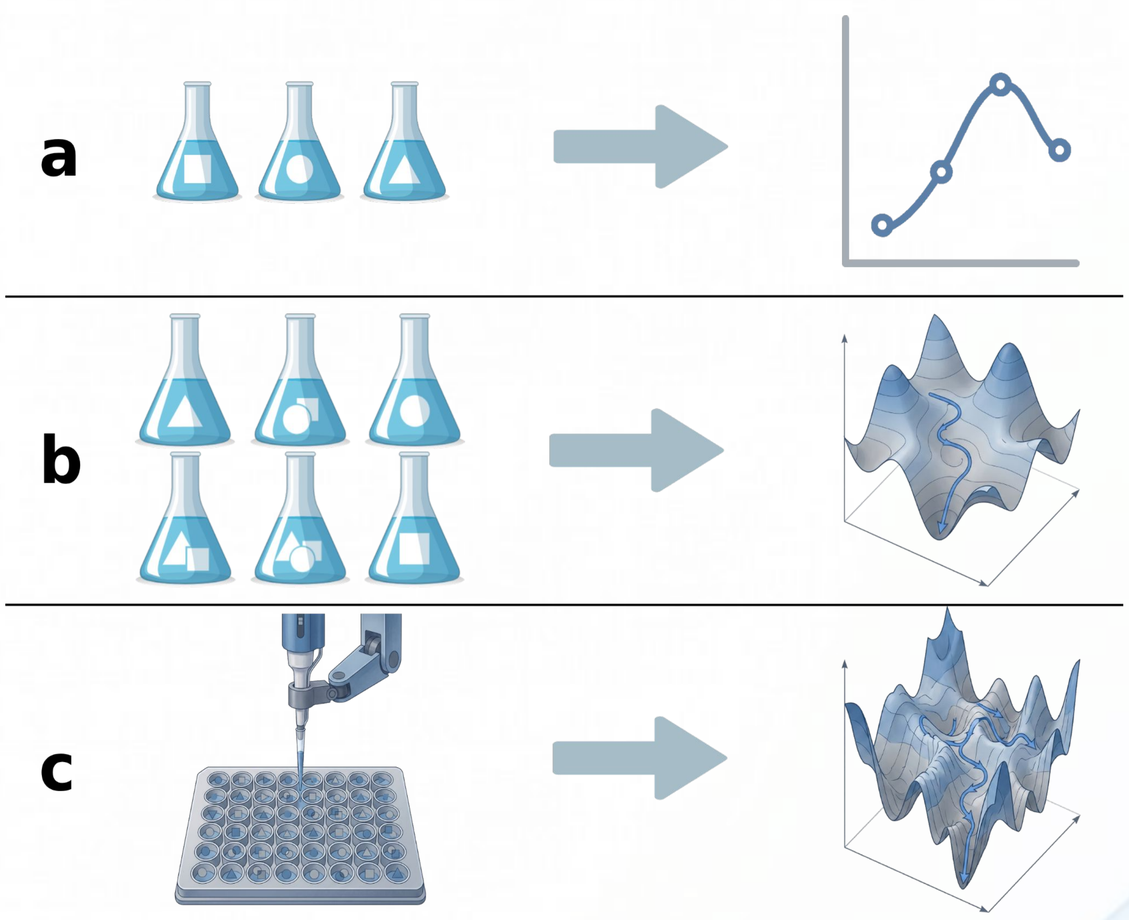


Figure 1. Different optimization approaches: **a.** One-factor-at-a-time, which focuses on changing variables one by one, neglecting the interaction of different factors, **b.** Design of Experiments, where multiple factors are optimized together, and the interaction of factors is considered partially, and **c.** Automated Batch Bayesian Optimization, where multiple factors are optimized simultaneously, and complex interactions of factors are taken into account.

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.

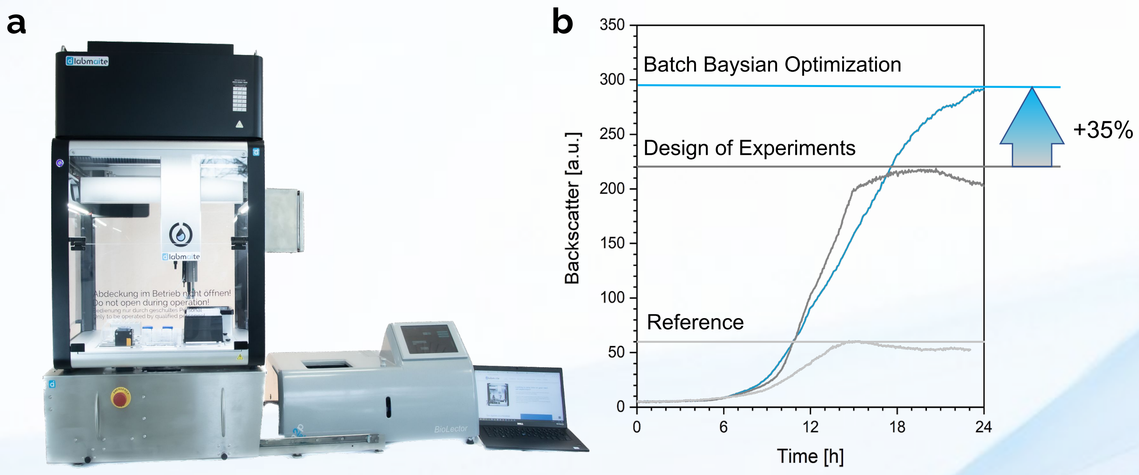
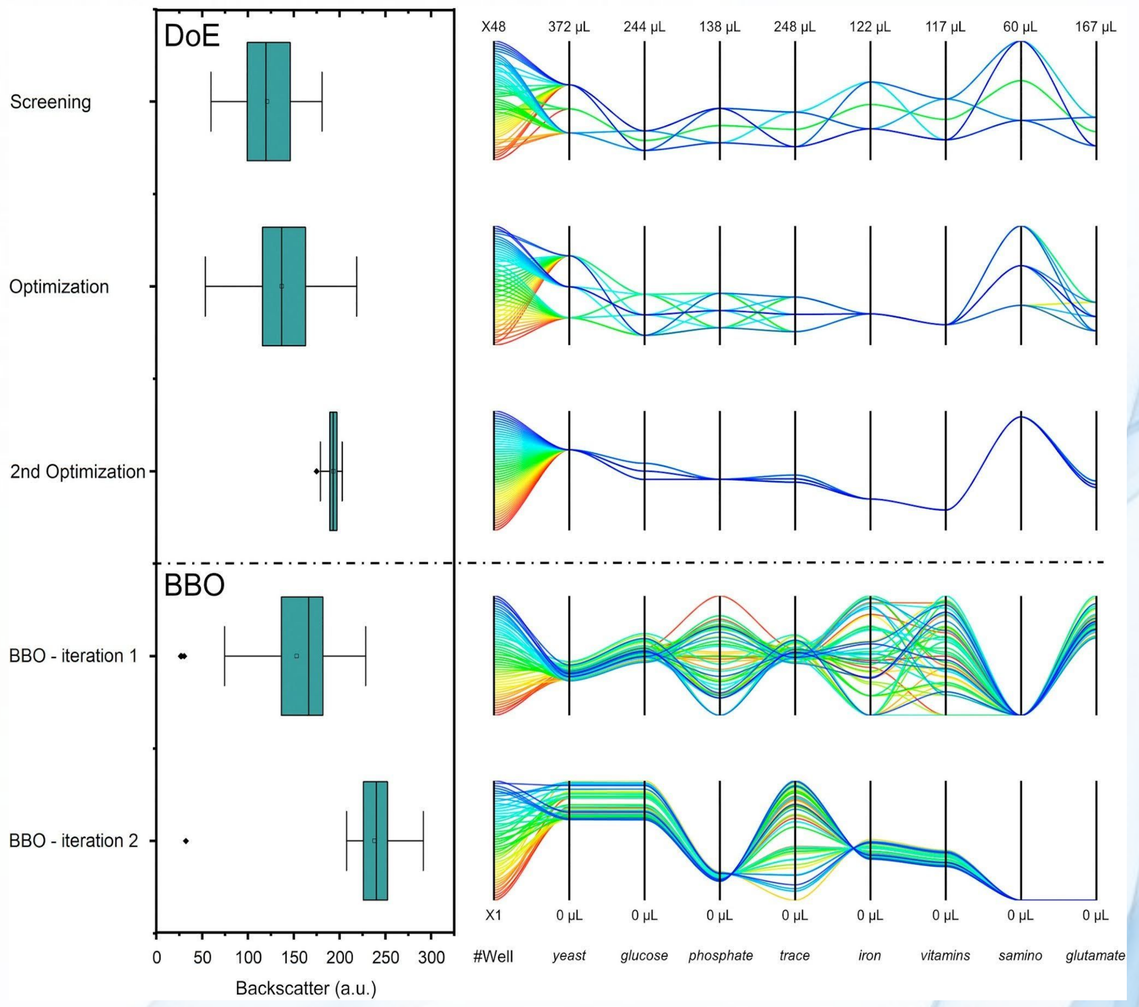


Figure 2. a. Automated cultivation device including BioLector I system, liquid handling system (OT-2), and turbidostat system. Right panel (b): Growth data for the microbioreactor cultivation of bacterium *Sporosarcina pasteurii*.

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.

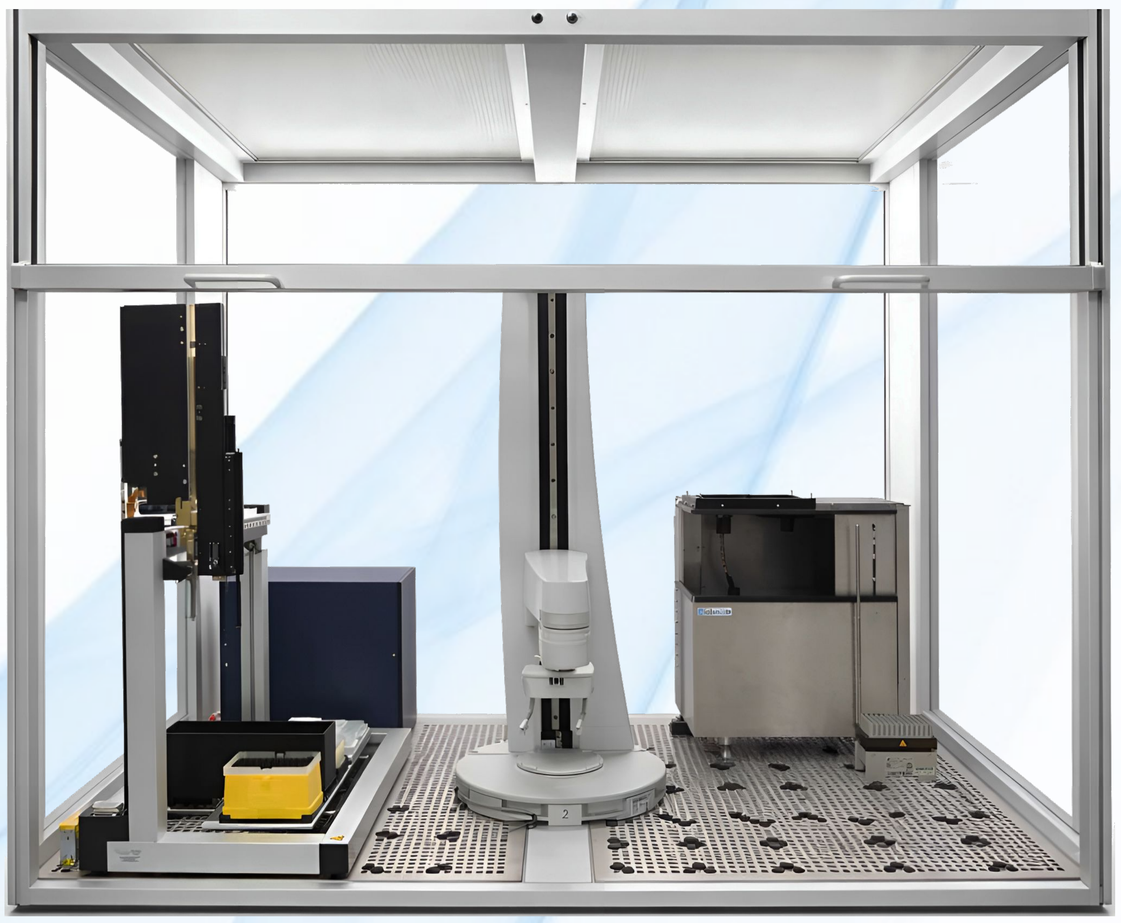


DoE vs. BBO variable exploration and optimization results. As shown above, BBO explores the factors to a greater extent than DoE, which explores only a few values for each component. The graph also shows BBO's superior performance over the same number of optimization runs.

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 plate handler, microscope, pipetting robot, and incubator at the hardware level, equipped with experiment control, automated data acquisition, AI analysis, and BBO optimization at the software level.

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