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# Amino Acid Fermentation: Evaluation of Scale-Down Capabilities Using DASbox® Mini Bioreactors

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## Abstract

Optimization of bioprocesses needs accurate monitoring and control while small working volumes are saving media and other resources. Evonik® has established a 2 L process for production of a nutritionally relevant amino acid in *E. coli*. The following application note descripes how this process was adapted to the smaller working volume of an Eppendorf DASbox Mini Bioreactor System.

Multiple runs were performed with close monitoring of all relevant process parameters and comprehensive evaluation of data to prove reliable and reproducible results. The scale-down capabilities of the DASbox system were evaluated by comparing the fermentation results to the data collected at 2 L scale.

## Introduction

Rising cost and time pressures in bioprocess development together with rapidly evolving regulatory requirements make process development efforts a special challenge these days. Optimizing every step of the total development workflow is crucial for maintaining a competitive business.

Advanced miniaturized benchtop bioreactor systems can harmonize operations between development and production groups while supporting the aims of Quality by Design (QbD). To meet today's demands of process development these mini bioreactor systems need to mimic all aspects of large-scale fermentation, and offer comprehensive data and information management tools to support regulatory requirements for both filing support and QbD-driven process development. *In situ* sensors as well as an integrated supervisory control and data acquisition (SCADA) are used to control, monitor, and record critical process parameters such as temperature, pH, dissolved oxygen, and agitation. As in production-scale bioreactors, gassing and feeding proceed according to defined settings. DASGIP® Parallel Bioreactor Systems have the potential to address process

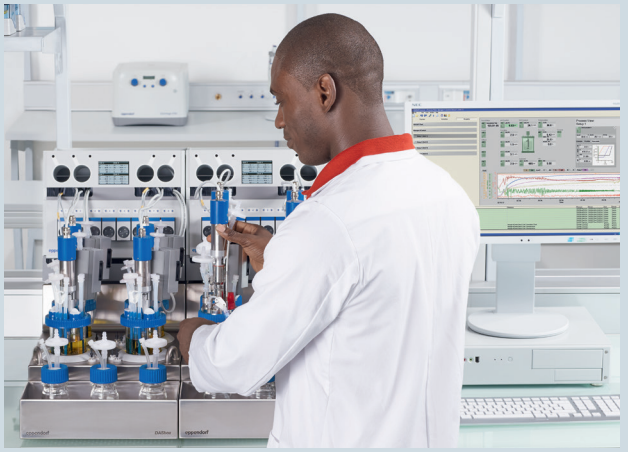

Figure 1: DASbox® Mini Bioreactor System for Microbial Applications

consistency and harmonization of operations between development and production.

The following application note illustrates how the DASbox Mini Bioreactor System with its working volume range of 60 - 250 mL supports bioprocess development in microbial applications. Scale-down capabilities were investigated by transferring a 2 L fermentation process to a 10x smaller working volume in the DASbox system.

Evonik Industries AG (headquartered in Essen, Germany) is one of the world's leading specialty chemicals companies. Its Health & Nutrition Business Unit produces and markets essential amino acids, mainly for animal nutrition and for specialties for the pharmaceuticals industry.

## Materials and Methods

To evaluate the reproducibility and scale-down capabilities of the DASbox Mini Bioreactor System (Figure 1) experimental series with two different systems were carried out and compared.

Fed-batch fermentation of the amino acid-producing *E. coli* strain was performed in a standard benchtop bioreactor. The corresponding small-scale approaches were carried out in a DASbox System equipped with four DASGIP Mini Bioreactors.

The *E. coli* strain was cultivated at 36 °C. During fermentation glucose was added according to predefined feed profiles. Both systems used comparable feeding profiles, the one of the DASbox system being adapted to the smaller working volumes. The DO set-point was maintained by adjusted agitation speed. The bioreactors were equipped with two Rushton impellers each. The pH value was regulated to a constant value throughout the fermentation process.

The critical process parameters were monitored, controlled and visualized online while additionally, optical density ($OD_{600}$) and glucose concentration were entered manually for collective analysis and storage in a joint database. Product concentration was measured at the end of each run.

## Results and Discussion

All critical process parameters such as feeding profiles and impeller tip speed as well as pH, DO, and temperature set-points were successfully transferred from the 2 L scale to the DASbox Mini Bioreactor System. The two systems showed similar growth characteristics. Online measured Oxygen Transfer Rates (OTR) resulted in highly comparable curves indicating a successful scale-down (data not shown).

Comparison of parallel fermentation runs performed with the DASbox prove the results to be highly reproducible. OTR values of all four runs again followed highly similar curves. Same was observed for online parameters such as temperature, dissolved oxygen concentration and pH. Fermentation using the DASbox system resulted in product yields comparable to the ones achieved with the larger benchtop system (Figure 2). Again, data obtained from the four individual runs performed with the DASbox Mini

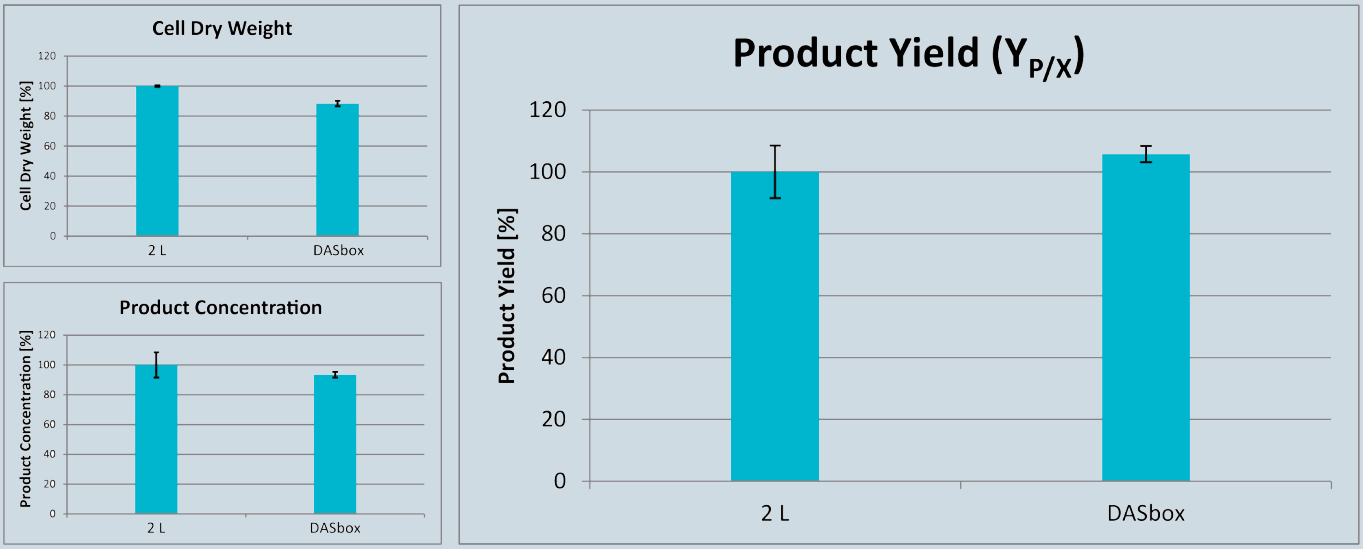


**Figure 2: Cell dry weight (X), product concentration (P) and specific product yield ($Y_{P/X}$), each normalized to the 2 L system.**

Bioreactor System strongly resembled each other and thus prove its reproducibility.

## Conclusion

The results presented in this application note give direct evidence to the scale-down capability of the DASbox Mini Bioreactor System. This proves the DASbox to be an excellent tool for microbial process development. With its small working volumes it helps saving resources without cutting back the comprehensive process control of advanced large-scale bioreactor systems. Summarized, the DASbox is a truly parallel mini bioreactor system that provides reliable and reproducible results.

| Ordering information | Order no. |
|---|---|
| **DASbox® Mini Bioreactor System for Microbial Applications** | |
| with 4 vessels, max. 25 sL/h gassing, for microbial applications | 76DX04MB |
| with 8 vessels, max. 25 sL/h gassing, for microbial applications | 76DX08MB |

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