

A Comparative Study: Small Scale *E. coli* Cultivation Using BioBLU® Single-Use and Reusable Vessels

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Abstract

In recent years single-use bioreactors gained more and more importance in animal and human cell culture. With the new line of BioBLU f rigid wall, stirred-tank single-use vessels Eppendorf offers premium solutions for microbial applications.

In the following case study, reproducible process control was achieved with parallel operated BioBLU 0.3f single-

use and reusable glass vessels, both used in an Eppendorf DASbox® Mini Bioreactor System. Fermentation of *E. coli* K12 led to very comparable results thus proving the tested single-use vessels to be an appropriate tool to accelerate microbial process development and shorten time-to-market in all industries related to microbial production processes.

Introduction

Single-use bioreactors are a suitable tool for time and cost effective bioprocessing. Minimized setup times, eliminated cleaning procedures and therefore reduced labor time can sustainably accelerate bioprocess development.

In all biopharmaceutical industries single-use technologies are widely used in mammalian cell culture. With the new BioBLU f line, specifically designed to meet the needs of fermentation, single-use bioreactors make their way to microbial applications in biopharma, food and cosmetics industries. Microbial applications make specific demands on the bioreactor design and functionality. Fermentation processes need much higher $k_L a$ values for proper mass transfer and suitable heating and cooling options as well.

This comparative study investigates the functionality and reliability of a BioBLU 0.3f single-use mini bioreactor and an autoclavable DASbox Mini Bioreactor (figure 2) in a small scale *E.coli* fermentation.



Figure 1: DASbox Mini Bioreactor System for microbial applications equipped with BioBLU 0.3f Single-Use Vessels and autoclavable DASbox Mini Bioreactors with Rushton-type impeller.



Figure 2: BioBLU 0.3f Single-Use Vessel (left) and DASbox Mini Bioreactor (right)

Materials and Methods

E. coli K12 (DSM 498) was cultivated in a fully instrumented Eppendorf BioBLU 0.3f single-use mini bioreactor and compared to fermentations in conventional autoclavable glass bioreactors.

The ready-to-use rigid wall stirred-tank single-use bioreactors, specifically designed for microbial applications, are equipped with a 2x Rushton-type impeller, liquid-free peltier exhaust condensation and direct drive for high performance agitation. The overhead-driven autoclavable DASbox Mini Bioreactors included 2x Rushton-type impeller and liquid free peltier exhaust condensers as well.

A 4-fold parallel DASbox Mini Bioreactor System with active heating and cooling capacities was used with DASGIP® Control* Software for precise process control.

Starting with a working volume of 100 mL each, the cultures were grown for 40 h in PAN medium with an initial glucose concentration of 40 g/L and fed with 50 % glucose solution in the fed batch phase.

The temperature was controlled at 37°C and pH was adjusted to 6.8 via 4 % ammonia solution; the cultures were submerged aerated with a constant rate of 1 vvm (6 sL/h or 0.1 sL/min). The dissolved oxygen was maintained at 30 %

with the stirrer speeds ranging from 600 rpm to 2000 rpm which equals to tip speeds of 0.94 m/s to 3.14 m/s. Exhaust concentrations were measured and corresponding oxygen transfer rates (OTR) were automatically calculated using a DASGIP GA4 exhaust analysis module.

Results and Discussion

Highly reproducible OTR values of up to 250 mmol/L/h were observed in the single-use as well as in the glass bioreactors (figure 3). Supporting $k_L a$ values of up to 2500 h⁻¹ were determined by static sulfite depletion method (data not shown) demonstrating that the single-use design of the BioBLU 0.3f bioreactors perfectly matches the demands of microbial applications.

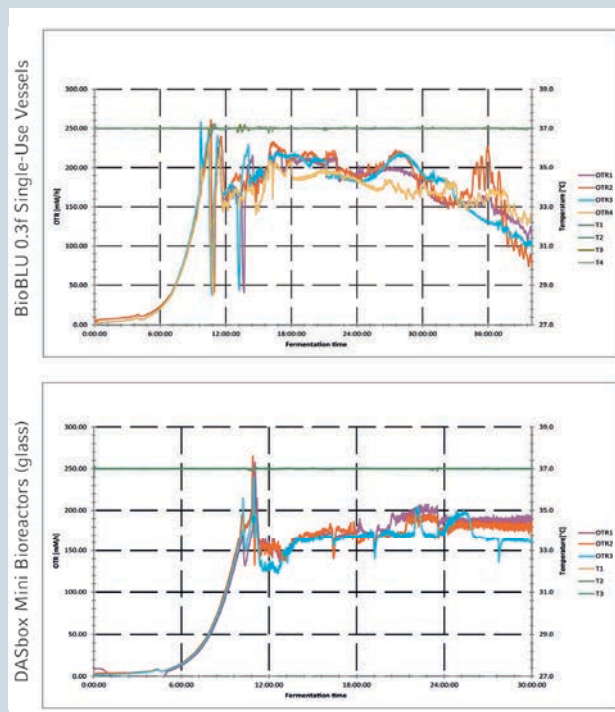


Figure 3: Reproducibility of online calculated oxygen transfer rates (OTR) of parallel process runs at constant temperature of 37 °C using BioBLU 0.3f single-use and reusable DASbox Mini Bioreactors, respectively. T = temperature, PV = process value

The biomass production was determined offline as cell wet weight and revealed comparable growth characteristics in single-use and glass mini bioreactors (figure 4). The maximal biomasses of about 160 g/L achieved in the fermentation are equal to an OD₆₀₀ of about 100 (data not shown).

*DASGIP Control is now DASware® control 5

Conclusion

This case study proves the BioBLU 0.3f single-use bioreactor addresses the specific needs of an *E. coli* fermentation especially in regard to mass and heat transfer. OTR values measured in the single-use vessel process runs as well as the final biomasses reached were comparable to those achieved with the conventional autoclavable DASbox glass bioreactors.

The specifically adapted single-use design of the BioBLU 0.3f mini bioreactor supports the high demands of microbial applications. Used with the Eppendorf DASbox this single-use bioreactor is a premium tool for screening, media optimization and as a scale down model for process development including Design of Experiments (DoE) approaches.

Industry interest in adequate single-use bioreactor solutions for fermentation is steadily increasing. With the BioBLU f line of single-use vessels Eppendorf is offering premium solutions for microbial applications. Users in fermentation can now benefit from advanced process control, accelerated process development, reduced costs and shorter time-to-market.

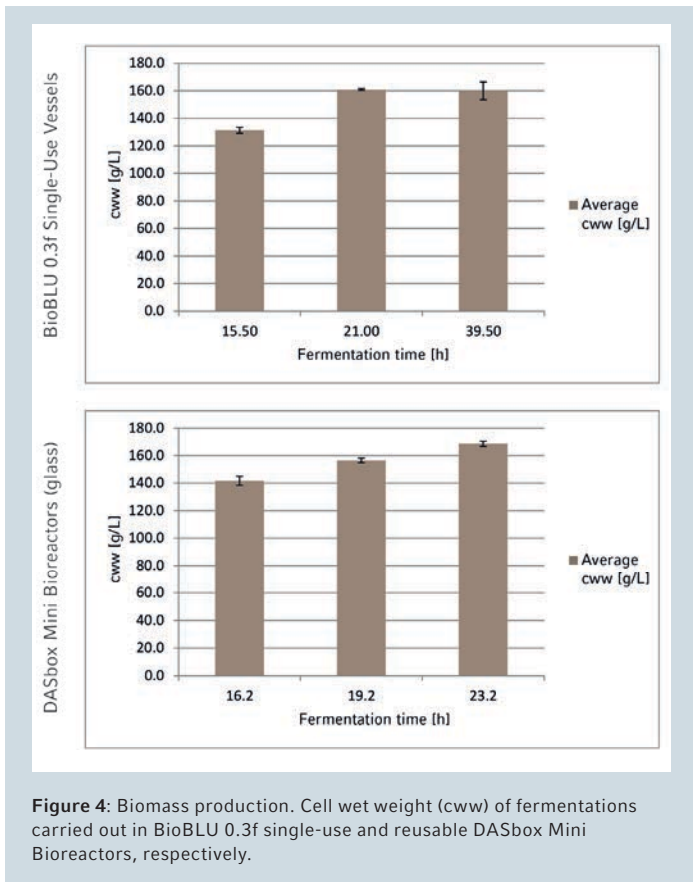


Figure 4: Biomass production. Cell wet weight (cww) of fermentations carried out in BioBLU 0.3f single-use and reusable DASbox Mini Bioreactors, respectively.

Ordering information	Order no.
DASbox® Mini Bioreactor System for Microbial Applications, max. 25 sL/h gassing	
4-fold system	76DX04MB
8-fold system	76DX08MB
16-fold system	76DX16MB
24-fold system	76DX24MB
4-fold system for single-use vessels	76DX04MBSU
8-fold system for single-use vessels	76DX08MBSU
16-fold system for single-use vessels	76DX16MBSU
24-fold system for single-use vessels	76DX24MBSU
DASbox® GA4 Exhaust Analyzing Module	
O ₂ 1 - 50 %, CO ₂ 0 - 25 %	76DXGA4
O ₂ 1 - 100 %, CO ₂ 0 - 25 % (GA4E)	76DXGA4E
DASbox® Vessel Type SR0250DLS	
2x Rushton-type impeller, 60 – 250 mL, overhead drive	76SR0250DLS
BioBLU® 0.3f Single-Use Vessels, microbial	
4 pack, pre-sterilized	78903509
DASbox® Exhaust Condenser, Peltier	
for 1 vessel	76DXCOND
for 1 single-use vessel	76DXCONDSU

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