APPLICATION NOTE No. 293 I October 2013

Scalability of Parallel *E. coli* Fermentations in BioBLU® f Single-use Bioreactors

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Abstract

Single-use bioreactor solutions have been successfully established in animal and human cell culture in the last years. Now this technology is going to make its way for microbial applications. In the following case study reproducible process control was achieved with single-use mini bioreactors and 1 L single-use vessels running in parallel. Fermentation of *E. coli* K12 led to highly reproduclible results thus proving the tested rigid wall single-use stirred-tank vessels to be an appropriate tool to accelerate microbial process development and shorten time-to-market in biopharmaceutical industry.

Introduction

Regardless if in cell culture or in microbial applications, single-use bioreactors provide a couple of advantages for time- and cost-effective bioprocessing. Minimal setup times, no need for cleaning procedures and therefore reduced labor time can accelerate bioprocess development rigorously. Compared to the use of single-use bioreactors in cell culture, microbial applications make specific demands on bioreactor design and functionality. Fermentation processes need much higher k_La values for proper mass transfer as well as suitable heating and cooling options.

Materials and Methods

To evaluate the reliability of microbial fermentation processes using single-use technology *E. coli* K12 (DSM 498) was cultivated in a fully instrumented Eppendorf BioBLU 0.3f single-use mini bioreactor and compared to fermentations in BioBLU 1f single-use bioreactors. This rigid wall stirred-tank single-use bioreactors were

Figure 1: BioBLU 0.3f and BioBLU 1f Single-use Vessels for Microbial Applications

carries baffles as well. Both vessel types include a liquid-free Peltier exhaust condensation and magnetic-coupled overhead drive for high performance agitation.

A 4-fold parallel Eppendorf DASbox® Mini Bioreactor System was used with BioBLU 0.3f Single-use Vessels and the BioBLU 1f fermentations were carried out using a 4-fold DASGIP® Parallel Bioreactor System with DASGIP Bioblock. Both Systems feature active heating and cooling capacities. DASGIP Control* Software was used for precise process control.

The cultures were grown for 24 h in PAN media with an initial glucose concentration of 40 g/L and fed with 50 % glucose solution in the fed batch phase. The processes were started with working volumes of 0.1 L in BioBLU 0.3f and 0.7 L in BioBLU 1f Single-use Vessels, respectively. The temperature was controlled at 37 °C.

When using the BioBLU 0.3f vessels the pH was adjusted to 6.8 via 4 % ammonia solution. The cultures were submerged aerated through dip tubes with a constant rate of 6 sL/h (1 vvm). Dissolved oxygen was maintained at 30 % whereas the stirrer speeds ranged from 600 rpm to 2000 rpm which equals to tip speeds of 0.94 m/s to 3.14 m/s. When using the BioBLU 1f vessels the pH was adjusted to 6.8 via 25% ammonia solution. The cultures were submerged aerated through dip tubes with a constant rate of 42 sL/h (1 vvm). Dissolved oxygen was maintained at 30 % whereas the stirrer speeds ranged from 600 rpm to 1600 rpm which equals to tip speeds of 1.35 m/s to 3.59 m/s. Oxygen transfer rates (OTR) were automatically calculated via a DASGIP exhaust analyzer GA4.

Results and Discussion

A two-phase cultivation with automatic feed-start was sucessfully carried out. As shown by the dissolved oxygen the utilization of BioBLU single-use vessels in combina-

*DASGIP Control is now DASware® control 5.

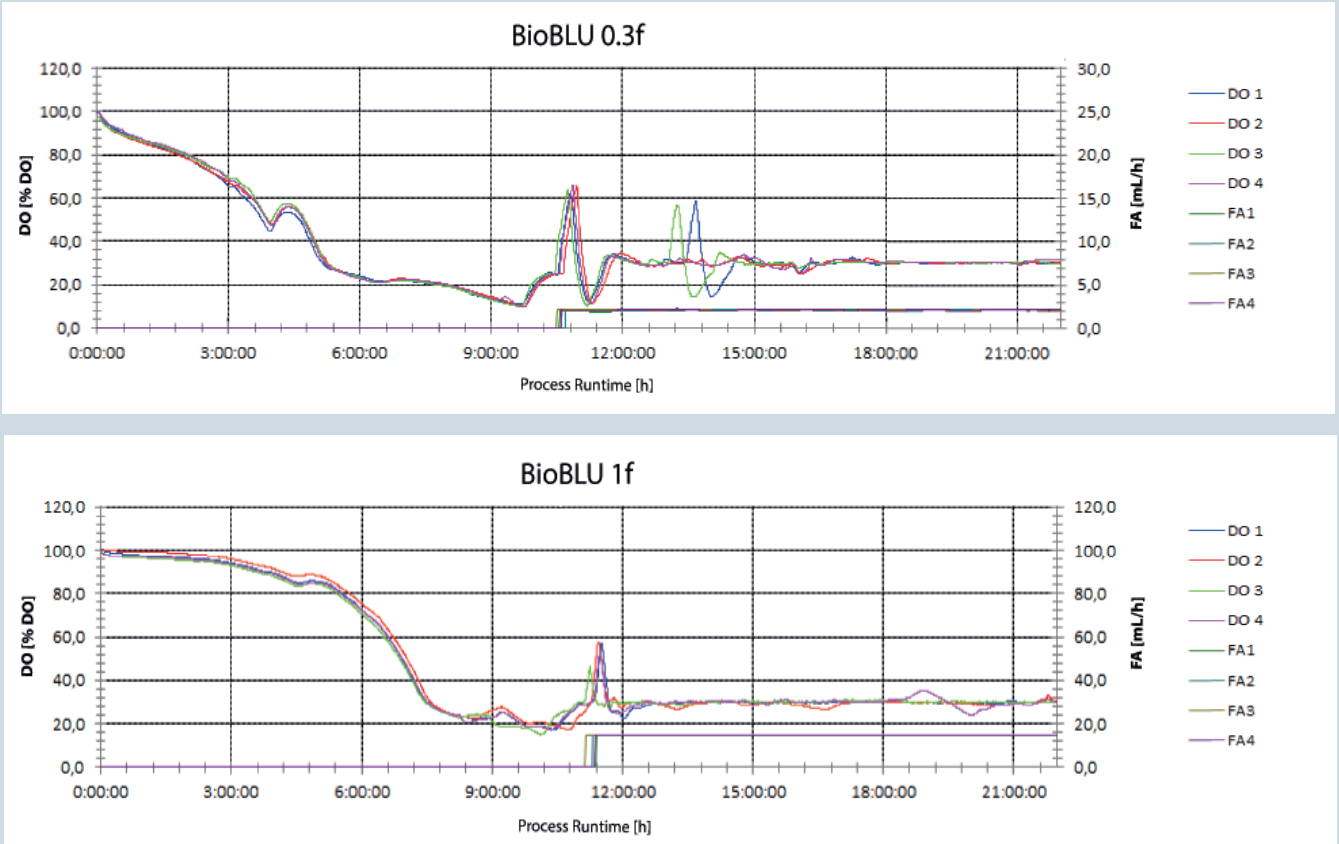

Figure 2: Parallel fermentation in BioBLU 0.3f and BioBLU 1f Single-use Vessels. The automatic feed-start was triggered by the glucose depletion induced DO peak in all four vessels in parallel. BioBLU 0.3f: 10.54 h ± 0.09 h after inoculation, BioBLU 1f: 11.31 h ± 0.11 h after inoculation. DO = dissolved oxygen concentration, FA = glucose pump rate.

tion with Eppendorf DASbox or DASGIP Bioblock allows highly parallel and reproducible fermentation (figure 2). Comparing the BioBLU 0.3f and the BioBLU 1f processes proves the capability for seamless scale-up from single-use mini bioreactors to 1 L single-use vessels. k_La values of up to 2500 h^{-1} in BioBLU 0.3f and up to 4000 h^{-1} when using BioBLU 1f vessels were determined by static sulfite depletion method (data not shown) and demonstrate that these single-use bioreactor designs perfectly match the demands of microbial applications. The biomass production (figure 3) was determined offline as cell wet weight and revealed comparable growth characteristics in both single-use bioreactors. The maximal biomasses of about 160 g/L gained in the fermentation runs correspond to an OD_{600} of about 100 (data not shown).

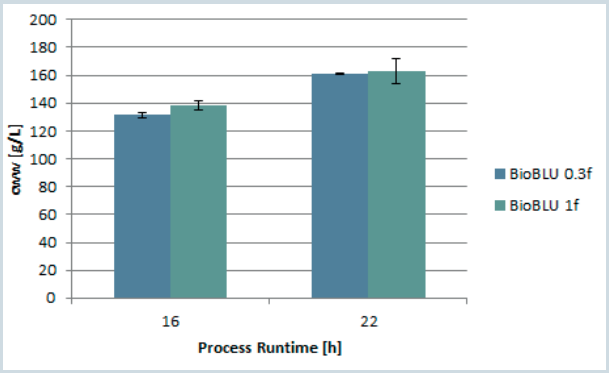

Figure 3: Biomass production. Cell wet weight (cww) of fermentations carried out in BioBLU 0.3f and BioBLU 1f Single-use Vessels, respectively.

Conclusion

This case study shows that the BioBLU f single-use bioreactors address the specific needs of *E. coli* fermentations especially in regard to mass and heat transfer. The specifically adapted single-use design, featuring Rushton-type impellers, active heating and cooling, and overhead drive enabling high performance agitation, supports the high demands of microbial applications. Currently, single-use bioreactor technology is mainly used in cell culture. With the introduction of the Eppendorf BioBLU f Single-use Vessels adequate tools to accelerate bioprocess development in microbial applications, even high cell density fermentation, are available now.

Ordering information	Order no.
DASbox® Mini Bioreactor System for Microbial Applications, max 25 sL/h gassing	
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
DASGIP® Parallel Bioreactor System for Microbial Applications, max. 250 sL/h gassing	
4-fold system with Bioblock, for single-use vessels	76DG04MBSU
8-fold system with Bioblock, for single-use vessels	76DG08MBSU
16-fold system with Bioblock, for single-use vessels	76DG16MBSU
BioBLU® 0.3f Single-Use Vessel, microbial, 4 pack, pre-sterilized	78903509
BioBLU® 1f Single-Use Vessel, microbial, 4 pack, pre-sterilized, 3 impellers	78903505

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