Application Note

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Univessel® SU Cultivation of CHO Cells in the Single-Use Bioreactor Univessel® SU

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Abstract

Over the past decade, single-use bioreactors have become widely accepted as alternatives to conventional stainless steel or glass bioreactors. In the biopharmaceutical industry glass bioreactors are used for instance as scale down models for process characterization. This is why it is extremely important that such vessels replicate the design of production-scale bioreactors for both reusable and single-use applications. This application note demonstrates that the 2 L Univessel® SU meets these criteria. Furthermore, it potentially increases the bioreactor controller run time by up to 25%. In this study, we demonstrate the successful batch cultivation of CHO DG44 in serum free, chemically defined media.

Find out more: www.sartorius.com/en/products/fermentation-bioreactors/benchtop-bioreactors/univessel-su

Introduction

Over the past decade, single-use bioreactors have become widely accepted as alternatives to conventional stainless steel or glass bioreactors for cultivation of mammalian cells in clinical manufacturing and process development. In the biopharmaceutical industry glass bioreactors are used mainly for process development and optimization, but also as scale down models for process characterization. This is why it is extremely important that such vessels replicate the design of production-scale bioreactors for both reusable and single-use applications. Stirred-tank bioreactors with 2-liter working volumes have proven to be particularly well-suited and deliver high performance for such applications. These models feature bioreactor geometries comparable to those of the production vessels, and their volume capacity is both affordable and sufficiently large for taking analytical samples. Above all, they are easy to use.

Advantages of Single-Use Bench-Scale Bioreactors

Beyond meeting the design criteria described above, single-use bioreactors eliminate the need for autoclaving relatively cumbersome glass vessels as well as the associated costs for their maintenance and repair. Moreover, Univessel® SU single-use culture vessels can also be used interchangeably with glass vessels so that during capacity peaks or maintenance of bioreactors, for instance, reusable vessels can be easily exchanged for single-use bioreactors in the interim. This enables the downtimes of the bioreactor controller to be reduced to an absolute minimum. Table 1 shows an example in which the run time of a bioreactor controller can be increased by 25% if single-use bioreactors are used instead of glass vessels. The only other way of attaining such high capacity utilization for a bioreactor controller is to purchase additional, fully-equipped glass bioreactor vessels, which entails high investment costs.

Univessel® SU Design

The Univessel® SU is a single-use bioreactor that meets the design criteria of conventional glass, stainless steel and state-of-the-art larger scale single-use stirred tank bioreactors. The culture vessel of the Univessel® SU is supplied as a pre-sterilized unit and is therefore ready to use right out of the box. It is fully assembled with impellers, a sparger and all the required tubing, filters and connectors. The exhaust line features a dual parallel-filter assembly, and all additional tubing can be conveniently attached to the vessel lid to maintain an orderly workspace.

Moreover, the Univessel® SU comes standard with integrated, non-invasive single-use pH and dissolved oxygen (DO) sensors. These sensors contain special dyes that are excited to fluorescence when exposed to light emitted through the culture vessel wall at a characteristic wavelength. The properties of the fluorescent light emitted by the sensor patches are influenced by the pH value or DO saturation of the culture medium that is continuously in contact with the sensor patches. The optoelectronics built into the culture vessel holder are used both to excite the dyes to fluorescence and to detect fluorescent light emitted.

Comparability and Connectivity

The Univessel® SU can be easily integrated into both new and existing bioreactor controllers. For pH and DO measurement, classic sensors or integrated single-use sensors can be used. The Univessel® SU Connection Box even makes it possible to utilize integrated single-use sensors with an existing bioreactor controller. The Connection Box is designed to align the pH and DO measuring path of the bioreactor controller via setting the reference value for calibration, as well as for entering calibration data for single-use sensors. The sensor calibration data can be input either manually or automatically by a barcode scanner that is even faster. As a result, this eliminates the need for time-consuming and labor-intensive steps involving sensor maintenance, autoclaving and installation.

Batch Cultivation of a Recombinant CHO Cell Line in a Univessel® SU

Cultivation Parameters

Bioreactor controller	Biostat® B-DCU II
Temperature	36.8 °C
Agitation seed	354 rpm (1 m/s)
DO	60% (controlled by N_2, air and O_2)
pH	7.15 (controlled by CO_2)

This case study demonstrates the performance of the Univessel® SU in batch cultivation of CHO with serum-free media, PowerCHO$_2$ (Sartorius Stedim Biotech GmbH | Lonza + 4 mmol glutamine). The particular clone used for this case study is an in-house generated CHO (DG44 ST1-C6) cell line expressing a human IgG1 antibody. Typically, it reaches cell densities between 6 – 7 × 10^6 cells/mL when grown in stirred-tank bioreactors.

Preparation of the Seed Culture

Cells were thawed, centrifuged and washed with media to remove residual freeze medium. Then they were transferred to a 500 ml Erlenmeyer flask (Corning) and incubated at 36.8 °C, 7.5% CO_2, 85% humidity and 120 rpm with an amplitude of 5 cm for 72 hr.

Setup for Cultivation

The Univessel® SU was filled two-thirds with medium, and the autoclaved, calibrated pH and DO sensors of a conventional design were installed under laminar flow. Afterwards, the culture vessel was placed in the vessel holder, and all necessary connections were made by a BioWelder tube welder. The filter heater was installed to prevent condensation in the exhaust filter. Subsequently, the DO sensor was calibrated. The temperature, pH and DO control loops were switched on to reach the set points. The bioreactor controller and the cultivation conditions used are summarized in the "Cultivation Parameters" box.

Inoculation of the Univessel® SU and Batch Run

Before inoculation, a sample was taken to verify its sterility. The cells were transferred to the Univessel® SU and, finally, the vessel was filled up to the maximum working volume of 2 L, resulting in a seed cell density of 0.25 × 10^6 cells/mL. At regular intervals, samples were taken using the aseptic sampling device on the Univessel® SU. Samples were analyzed for viable cell density and viability using a Cedex analyzer. Glucose and lactate concentrations were each determined. Figure 2 shows the viable cell density and the viability and Figure 3 the corresponding glucose and lactate concentrations over the course of 10-day batch cultivation.

Results and Discussion

In the Univessel® SU, the cells grew well up to a cell density of 6.8 × 10^6 cells/mL after 191 hr, maintaining a viability of ~ 90% until the nutrient source, glucose, was depleted after 238 hr of batch age, which indicated the end of cultivation. In conjunction with glucose consumption, the build-up of lactate could be observed. As the glucose concentration decreased, the cells started to use up lactate as a secondary carbon source after 112 hr.

Conclusion

The Univessel® SU is a 2 L working volume single-use bioreactor that meets the design criteria of conventional glass bioreactors. Furthermore, it potentially increases the bioreactor controller run time by up to 25%. In this study, we have demonstrated the successful batch cultivation of CHO DG44 in serum free, chemically defined media. Cell densities of of 6.8 × 10^6 cells/mL, which are typical for the particular clone when grown in a conventional bioreactor, could easily be reached while maintaining good viability.

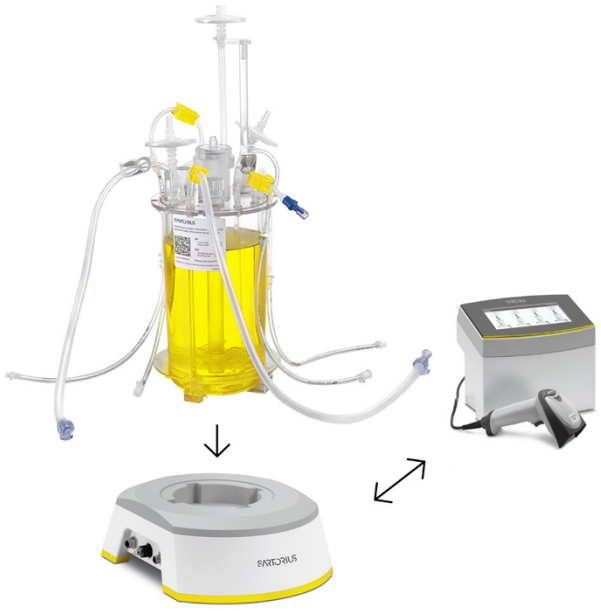

Figure 1: *Univessel® SU With Holder and Connection Box*

Process step	Glass bioreactor	Single-use bioreactor Univessel® SU
Pre- and after run preparation time (Vessel assembly, sensor calibration, autoclaving, medium fill, harvest, cleaning)	2 days	~ 1 hr
Sterility test	1 day	None
Culture time	12 days	12 days
Possible runs per year per bioreactor controller	24	30 (+25%)

Table 1: *Comparing Single-Use and Glass Bench-Top Bioreactors*

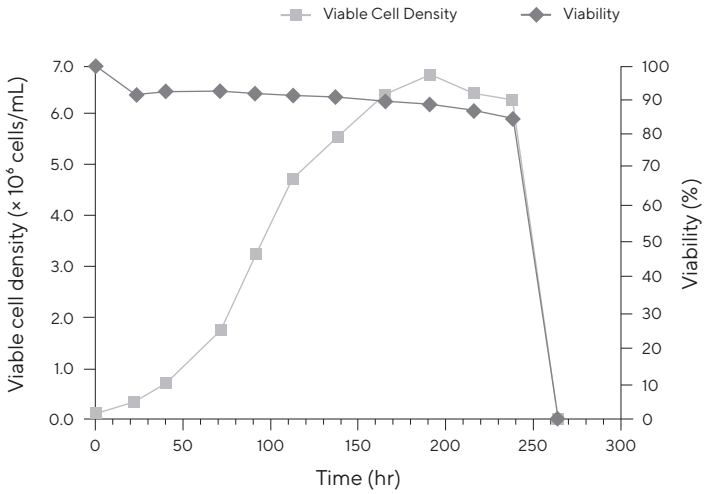

Figure 2: *Viable Cell Density and Viability*

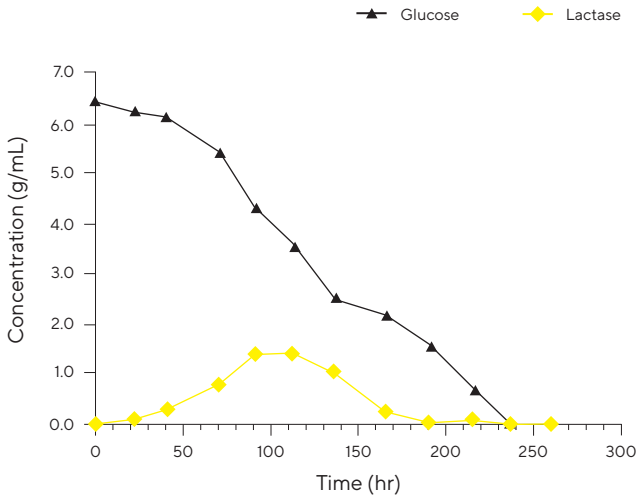

Figure 3: *Analysis of Glucose and Lactate Concentration*

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