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Biostat® CultiBag STR

Application Note

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Large scale cultivation of CHO cells in the stirred single-use bioreactor Biostat® CultiBag STR

turning science into solutions

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Background

The Sartorius Stedim Biotech Biostat® CultiBag STR is the first and only single-use bioreactor which was engineered based on the well-established principles known from classical reusable bioreactors.

Single-use bioreactors are nowadays widely accepted in research and manufacturing applications of mainly mammalian and insect cell cultures. They have several advantages compared to traditional stainless steel assemblies. Conventional production plants are commonly dedicated to a specific product. In contrast, single-use bioreactors are tremendously flexible because all piping is realized in the disposable bag, which can be designed according to the application. Costs are reduced by eliminating the space consuming and expensive CIP and SIP installations and reducing the needed production turnaround times. In combination with the reduced efforts for validation, this eventually results in a shorter time to market and a faster return on investment. On the other hand, stainless steel bioreactors rely on proven engineering principles. For decades, stirred tank bioreactors featuring a cylindrical vessel, stirred by impellers mounted on a rigid shaft and gassed via a submerged sparger, have been the gold standard. They are well characterized in terms of performance, scalability, optimization steps and design.

In single-use bioreactors, pre sterilized single-use bags of numerous types and shapes are used as cultivation chambers. Most single-use bioreactors rely on different design principles as their stainless steel counterparts, making process transfer or scale up calculations from a single-use to a stainless steel reactor a challenge. While most of these single-use bioreactors are per se well suited for the cultivation of mammalian or insect cells, knowledge and experience gained in stainless steel stirred tank reactor processes is difficult to apply. Scaling up single-used bioreactors from a smaller clinical phase scale to a commercial scale production process, which will today still be run in a several cubic meter stainless steel bioreactor, is a more challenging task. Also, their use in a hybrid plant featuring fully scalable single-use and reusable bioreactors is somewhat limited.

While taking these limitations of current single-use bioreactors into account, Sartorius Stedim Biotech has engineered the Biostat® CultiBag STR plus 200 as shown in figure 1. This system is the only single use bioreactor with the exact geometries and features of a classical reusable stirred bioreactor. This allows an easy transfer from reusable to single-use processes. Furthermore it allows the user to move very easily from a single-use bioreactor to a stainless steel conventional bioreactor without any additional optimization steps. Such necessity occurs in case the maximum scale of the used single-use bioreactor (for example 1000 L) is only an intermediate step in the process and inoculation into a 10,000 L stainless steel reactor is required.

Figure 1: Biostat® CultiBag STR plus 200

The design of the disposable bioreactor bag is based on the geometries of conventional vessels with a cylindrical shape, a height to diameter ratio of 2:1 and a convex bottom with the harvesting port at the lowest position. For agitation, a choice of classical 3-blade segmented impellers or 6-blade rushton turbines, mounted on a rigid impeller shaft, is available. The shaft is pre-installed into the bag and coupled to the drive motor via a magnetic coupling. Mixing is fast and homogenous. Classical micro- or ringspargers are standard in the CultiBag STR and are installed below the stirrer device for an efficient gas transfer. The Biostat® CultiBag STR is equipped with a flexible, feedback controlled gassing system which can change the composition of the gas. The composition of air, O_2, N_2 and CO_2 is automatically determined based on the feedback coming from the DO and pH sensors. All gasses can either be directed to the sparger or the overlay.

All components in contact with the media are designed for single use. The bag is made out of Stedim 40 film with ULDPE as the contact layer. The stirrer, impellers and sparger are made out of polycarbonate. Single-use, pre-installed pH and DO probes are used. They rely on a fluorescence based measurement principle which uses contact free signal transmission via a fibre optical cable. All materials USP class VI compliant.

In this application note, we demonstrate the performance of the Biostat® CultiBag STR in the batch mode cultivation of CHO in a culture volume of 200 L. Serum free media was used for the cultivation. The particular CHO clone used for this study is the in house generated DG44 ST1-C6 expressing a human IgG. Typically, it reaches cell densities between 6-7 × 10^6 cells/mL when grown in bioreactors in this type of media.

Material

- Biostat® CultiBag STR 200 Plus single use bioreactor (Sartorius Stedim Biotech)
- CultiBag STR 200 disposable bioreactor bag (Sartorius Stedim Biotech) containing 2 × 3-blade segmented impeller top to bottom flow, microsparger 22–45 µm pore size.
- ProCho5 medium (Lonza), 4 mM L-glutamine, 1 × hypoxanthine|thymidine, supplied in 100 L containers.
- Cell line CHO-DG44 clone ST1-C6, generated in house, producing a human IgG.
- BioWelder tube welder for thermoplastic tubes (Sartorius Stedim Biotech)
- BioSealer tube sealer for thermoplastic tubes (Sartorius Stedim Biotech)
- Biostat® CultiBag RM single use bioreactor (Sartorius Stedim Biotech)
- CultiBag RM 20 L optical disposable bioreactor bag (Sartorius Stedim Biotech)
- Nucleocounter (Sartorius Stedim Biotech)

Preparation of seed culture

The CHO clone was expanded in ProCHO5 Serum free medium over several steps from the WCB to 10 L culture volume in a rocking motion single use bioreactor bag, the Biostat® CultiBag RM 20 optical equipped with a CultiBag RM 20 L disposable bag. Cells were grown at 37°C, pH 7.1, 40% DO until a viable cell density of 3 × 10^6 cells/mL was reached.

Setup for cultivation

A container holding 5 L of 1 M NaOH was connected to the CultiBag STR under laminar flow using a CPC connection. Afterwards, the bioreactor bag with the connected gas filters was installed into the bag holder and the gassing lines were connected to the control tower and the bag inflated. A filter heater was installed to prevent condensation in the exhaust filter. The sensors were connected to the measurement amplifiers of the control unit using optical fibers and sensor calibration data were entered into the control system.

Using the BioWelder, a connection between two C-Flex®* tubings from the media hold container and the bioreactor bag was made and 50 L of media were transferred into the CultiBag STR. The tubes were disconnected using the BioSealer. The temperature control loop was set to 37°C and heating was switched on. The media was incubated over night to provide a sterility test.

Inoculation of CultiBag STR and batch run

The reactor pH and DO control loops were switched on to reach setpoints of pH 7.1 and 40% DO. pH was controlled by the addition of NaOH via a peristaltic pump and CO_2 via sparger. DO was feedback controlled by addition of N_2, air and O_2 via sparger. Aeration was switched on at maximum 0.01 vvm. The stirrer was switched on at 100 rpm corresponding to a tip speed of 1.2 m/s.

Before inoculation, a sterility sample was taken to prove sterility. After the set points were reached, a connection of the harvest line from the CultiBag RM was made to an addition line of the CultiBag STR using a BioWelder. After transfer, the line was disconnected using the BioSealer. The seed inoculum was transferred into the CultiBag STR, leading to a seed cell density of 0.57×10^6 cells/mL. The batch was run for approximately 6 days under the above described conditions. After 44 h, when the viable cell density had reached 4×10^6 cells/mL, the reactor volume was filled up with 140 L medium to a total volume of 200 L. The batch was continued until day 6. In regular intervals, samples were taken via the aseptic sampling device on the CultiBag STR. Samples were analysed for viable cell density and viability using the automated Nucleocounter device. Glucose and Lactate concentrations were determined. Figure 2 shows the viable cell density and the viability over the course of the 6 day batch culture. The nick at 44 hrs indicates the dilution of the cells by increasing the media volume. The cells grew well up to a final cell density of 6.3×10^6 cells/mL maintaining a viability of >90%. After 120 h of batch age, the viable cell concentration and viability started to decrease.

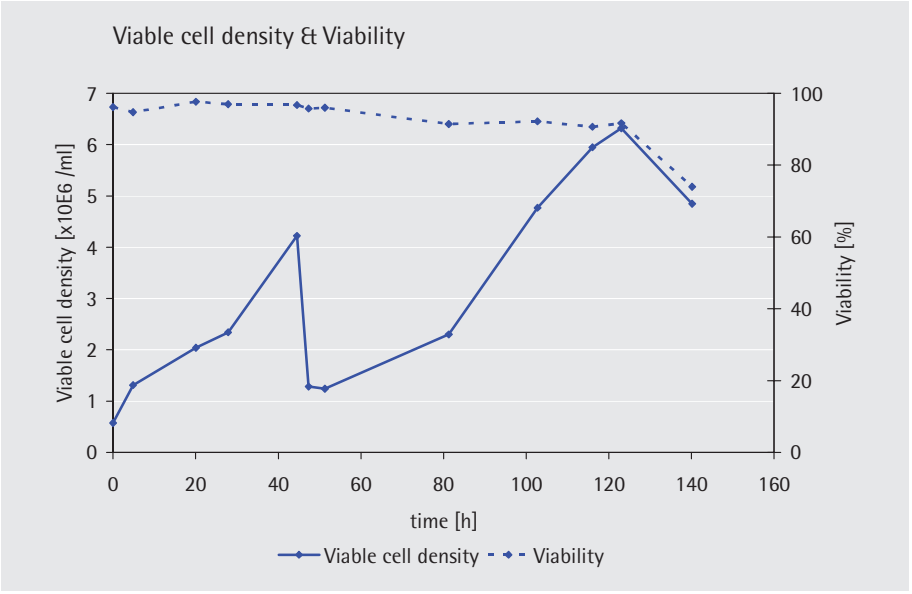

Figure 2: Viable cell density and viability during the batch cultivation

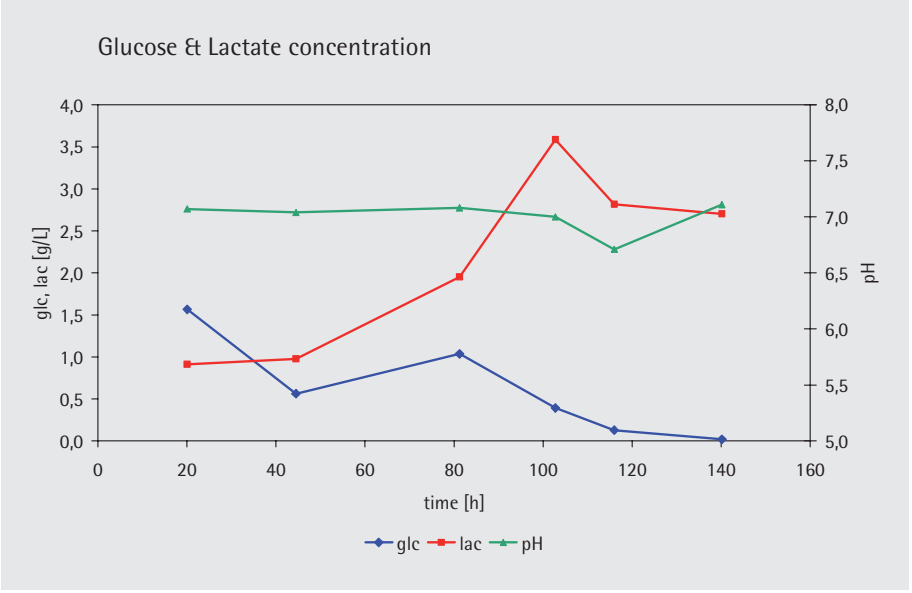

Figure 3: Analysis of lactate & glucose concentration, pH measurement.

* C-Flex® is a registered trademark of Saint-Gobain Performance Plastics Corporation.

As can be observed from figure 3, the glucose in the media was used up after 140 h of cultivation, indicating the end of the cultivation. In conjunction with glucose consumption, the build up of lactate can be observed, again the dent in the curves at around 44 h resulting from the dilution of the growth media.

Figure 3 also displays the pH at the corresponding time points measured online using the fluorescent disposable probes. As can be seen from the figure, acidification of the media by lactate build up was prevented by pH control. The drop of pH at around 120 h resulted from the emptying of the NaOH container. After installation of a new NaOH container, the pH was controlled back to the setpoint.

Conclusion

The Biostat® CultiBag STR is the only single-use bioreactor which is engineering completely on principles known from reusable bioreactors. It is completely comparable to conventional stainless steel bioreactors and fully scalable. In this study, we have demonstrated the successful batch cultivation of CHO DG44 in serum free, chemically defined media under tight feedback control of pH and DO. Cell densities of 6.3×10^6 cells/mL, which are typical for the particular clone when grown in a bioreactor, could easily be reached while maintaining good viability.

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