2,000 L HyPerforma Single-Use Bioreactor Evaluation

Scale-up and 2,000 L production scale of CHO cells using the Single-Use Bioreactor

Introduction

The stirred-tank reactor is the most commonly utilized reactor type in biotechnology. The Thermo Scientific™ HyPerforma™ Single-Use Bioreactor (S.U.B.) is designed as a stirred-tank reactor for animal cell culture and has the unique advantage of utilizing completely single-use product contact surfaces. This design is based on years of traditional successful stirred-tank reactor design and operation. Working volumes of 25 L to 2000 L are now supported using this technology.

During the design of each size of S.U.B., care and consideration were given to produce scalable systems capable of delivering consistent performance through the range of working volumes. Multiple factors are considered in selecting a scale-up technique, but generally include heat and gas transfer as well as mixing capabilities of the hardware systems. Since the S.U.B. is based upon the traditional stirred-tank reactor, many of the classical and well understood scale-up/scale-down strategies can be employed. Critical design parameters such as height-to-diameter ratios, agitation design, and control systems have been preserved, enabling the user to scale easily.

A monoclonal antibody producing CHO cell line was used to show performance characteristics of similar cell lines when grown in S.U.B.s. A completely single-use seed train was used to seed the 2000 L production- scale S.U.B., including 50, 250 and 1000 L S.U.B.s.

The culture was monitored for cell growth and viability at each stage of the seed train and at the final 2000L production scale. Cultures in each size of S.U.B. were used to seed the next scale in the process until reaching 2000 L, in which 2 x 2000 L S.U.B.s were seeded from a single 1000 L S.U.B. These two 2000 L S.U.B.s were used to run cultures in parallel in order to assess reproducibility of the system's performance.

Materials and methods

Cell culture medium and reagents

A CHO medium of sufficient quantity and packaging sizes to complete the campaign (approximately 5500 L), was prepared from powder by hydrating 17.2 g/L in WFI water at approximately 90 percent of the final volume (5500 L). Once the cell culture medium was fully dissolved, sodium bicarbonate (SV10007) was added at a concentration of 2.2 g/L and mixed until dissolved, then l-glutamine (SV10006) at 0.58 g/L was dissolved and finally pluronic (SV10141) was added at 1.0 g/L.

The solution was then brought to full volume and mixed for an additional 30 minutes. The solution was then filtered into gamma irradiated, sterile containers, including 5 x 1000 L, 2 x 200 L, 1 x 50 L, 2 x 20 L Thermo Scientific BioProcess Containers (BPCs) and 10x1 L bottles via 0.2 µm sterile filtration.

Cells, scale-up and production-scale process:
Chinese Hamster Ovary (CHO) cells used in this study were thawed from a cryopreserved cell bank and passaged on a 3-4 day schedule during scale-up, with seeding densities of approximately 2.5 x 10^5 viable cells/mL. Cells were cultured in batch mode only, without feeding.

The seed train consisted of the approximate culture volumes and vessels listed below:

- 1 x 10 mL working volume in a single-use, 125 mL Erlenmeyer shake flask
- 1 x 50 mL working volume in a single-use, 250 mL Erlenmeyer shake flask
- 1 x 200 mL working volume in a single-use, 500 mL Erlenmeyer shake flask
- 1 x 1 L working volume in a single-use, 2.8 L Fernbach shake flask
- 1 x 6 L working volume in a 14 L stirred-tank bioreactor
- 1 x 35 L working volume in a 50 L S.U.B. – Figure 1
- 1 x 200 L working volume in a 250 L S.U.B. – Figure 1
- 1 x 1000 L working volume in a 1000 L S.U.B. – Figure 1
- 2 x 2000 L working volume in 2000 L S.U.B.s – Figure 2

As evident from the seed train, a single 1000 L S.U.B. was used to seed two 2000 L S.U.B.s that were run in parallel. The systems of 1000 L or less are available in both liquid- jacketed and electrically-heated versions. The 2000 L systems are liquid-jacketed only. The liquid-jacketed versions require a separate temperature control unit, which is connected to the jacket via stainless steel flexible hosing and quick connect couplings. Bioreactor controllers were used to monitor and maintain dissolved oxygen (dO_2), pH, temperature and agitation set points.

Culture biochemistry, cell population density, and viability were monitored at least once daily. Although the non-fed batch cultures would typically be maintained for approximately 7 to 8 days, the 2000 L bioreactors were operated for a total of 21 days in order to assess sterility.

Figure 1. Thermo Scientific 50 L (left), 250 L (right), 1000 L (center) liquid-jacketed HyPerforma Single-Use Bioreactors.

Figure 2. Thermo Scientific 2000 L HyPerforma Single-Use Bioreactor.

Results and discussion

Achieving scale-up to 2000 L resulted without issue and was found to be simple. Cells exhibited typical growth and viability throughout the process. Figure 3 shows cell growth in each of the S.U.B.s during scale-up, including the parallel cultures maintained at the 2000 L production scale. Cells reached approximately 5.0 to 5.5x106 cells/mL at each passage (every 3 to 4 days) and reached a maximum viable cell population density (VCPD) of approximately 6.5x106 viable cells/mL.

The parallel cultures at the 2000 L scale are nearly identical in growth performance and demonstrate the reproducibility of results using the 2000 L S.U.B. In addition, all cell growth curves throughout the scale-up process show consistent growth of the CHO cells in each size of S.U.B. Figure 4 depicts the cell growth and viability curves overlaid in order to assess the consistency and scalability of these systems.

Metabolite profiles for the 2000 L cultures are shown in Figure 5. Samples were collected for analysis beginning at seeding, or time zero. The profiles for pH, glutamine/ ammonium, and glucose/ lactate were nearly identical over the 7 day culture period for both bioreactor cultures, adding further evidence to the reproducibility of performance using the system. Although cell count, viability and metabolic analyses were discontinued after day 7, the bioreactors continued to operate, maintaining temperature, agitation and pH setpoints for a total of 21 days during which time the systems were monitored for any sign of contamination. No contamination occurred throughout the 21 day period.

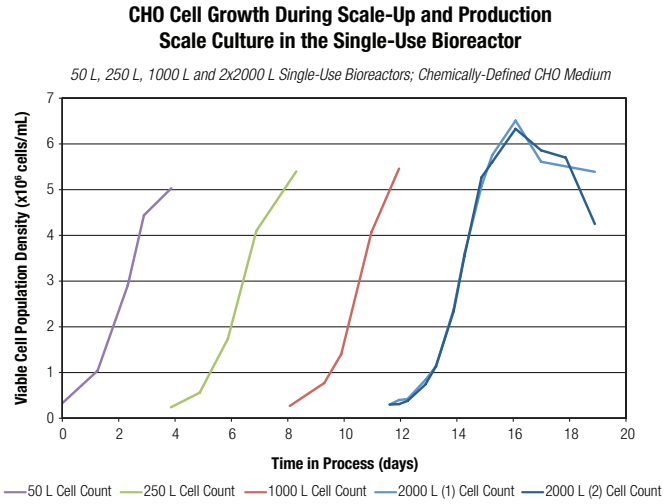

Figure 3. CHO cell growth during scale-up and production stage in Thermo Scientific HyPerforma Single-Use Bioreactors (S.U.B.s) of 50, 250, 1000 and 2000 L volumes.

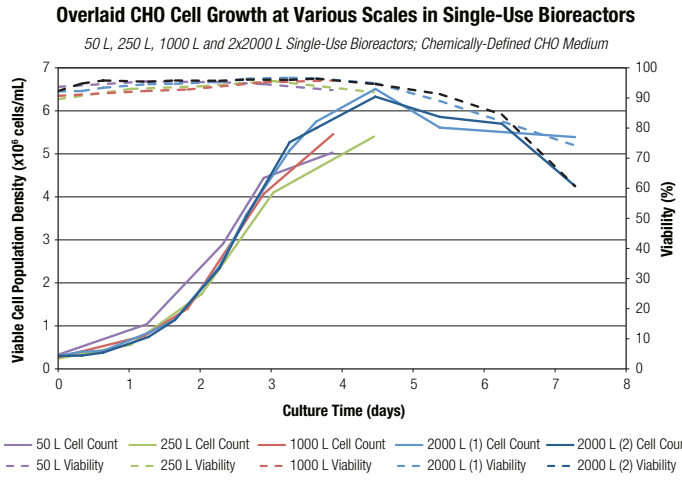

Figure 4. CHO cell growth and viability curves from all Thermo Scientific HyPerforma Single-Use Bioreactor (S.U.B.) cultures overlaid demonstrating the consistent and scalable performance of the systems.

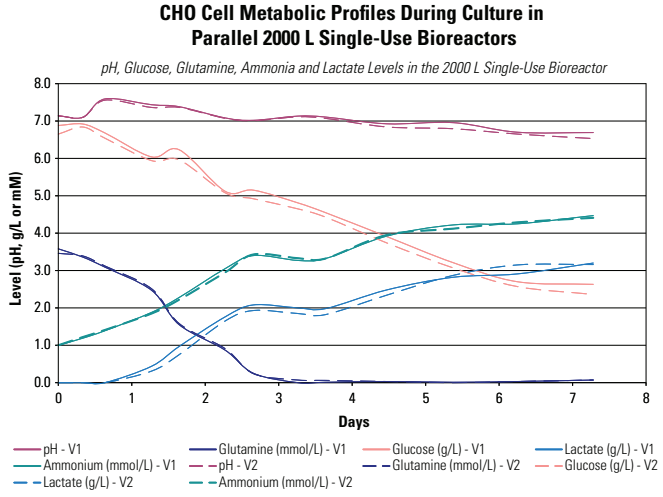

Figure 5. Metabolic profiles of parallel CHO cells cultured in 2000 L Thermo Scientific HyPerforma Single-Use Bioreactors (S.U.B.s).

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Summary

A CHO cell culture was scaled up through 50, 250 and 1000 L S.U.B.s in order to seed 2x2000 L S.U.B.s in parallel. The scale-up and 2000 L production scale runs were successful and were operated for 21 days, monitoring cell growth, viability, metabolic profile and evidence of contamination. Results from these runs show a high degree of scalability with respect to cell growth and viability among the various sizes of S.U.B. Additionally, the 2000 L production scale cultures run in parallel exhibited nearly identical biochemical profiles, including pH, and metabolism of glucose, lactate, glutamine and ammonium. The Thermo Scientific S.U.B. allows efficient scale-up and reproducible performance through the 2000 L scale based upon CHO cell growth, viability and general metabolism.

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