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Scale-up of fed-batch process utilizing Dynamis Medium and EfficientFeed C+ Supplement in HyPerforma S.U.B.s

Abstract

The purpose of this work was to demonstrate the scalability of a fed-batch process of CHO cells in Gibco™ Dynamis™ Medium with Gibco™ EfficientFeed™ C+ Supplement, utilizing a pH shift strategy. Thermo Scientific™ HyPerforma™ Single-Use Bioreactors (S.U.B.s) were operated with a variable CO_2 cascade which, along with the buffering capacity of the medium, provided a reproducible pH shift profile up to the 250 L scale.

Introduction

One key factor for ensuring comparable growth and productivity during cell culture scale-up is pH control. pH impacts many factors in this process, including specific productivity, cell growth, and cell metabolism. The buffering capacity of the medium and feed is important in controlling pH and therefore developing a scalable and reproducible process.

For this work, we leveraged a pH shift, continuous feeds of EfficientFeed C+ Supplement and glucose, and the parameters outlined in Table 1. This approach resulted in a reproducible lactate profile and enabled consistent cell growth and productivity in 5 L to 250 L bioreactors.

Materials and methods

The Gibco™ Freedom™ CHO-S™ cell line producing a recombinant antibody was recovered from cryopreservation and expanded in Dynamis Medium.

Cell concentration and viability were measured on the Vi-CELL™ Cell Counter (Beckman Coulter); antibody titer was measured on the Cedex Bio HT analyzer (Roche) and normalized to shake flask titer on day 14. pH, pressure of dissolved CO_2 (pCO_2), and lactate concentration were measured on the BioProfile FLEX™ Analyzer (Nova Biomedical).

The pH shift was managed by a CO_2 cascade through the drilled-hole sparger without addition of any base. Additionally, the dissolved oxygen (DO) setpoint was maintained through an O_2 cascade through the drilled-hole sparger.

Table 1. Operating parameters for vessels.

Parameters	500 mL shake flask	5 L glass bioreactor	50 L HyPerforma S.U.B.	250 L HyPerforma S.U.B.
Inoculation volume	100 mL	4.25 L	42.5 L	212.5 L
Inoculation density	0.3 x 10^6 vc/mL			
2X EfficientFeed C+ Supplement	5% each on days 3, 5, 7	Continuous and constant on days 3–10, 15% total		
Glucose (45% solution)	Bolus as needed	Continuous and constant		
pH control	8% CO_2	Variable CO_2 sparge		
Temperature	37°C			
Dissolved O_2	NA	30% saturation		
Agitation	150 rpm	200 rpm (pitched blade)	178 rpm (pitched blade)	122 rpm (pitched blade)
Overlay	NA	0.15 L/min	2 L/min	4 L/min
Sparge	NA	Variable O_2		

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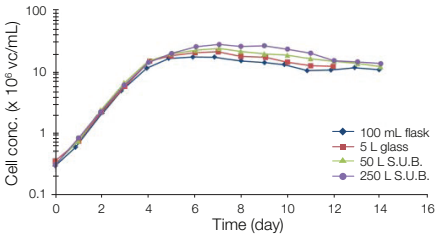

Figure 1. Cell growth across scales in Dynamis Medium and EfficientFeed C+ Supplement.

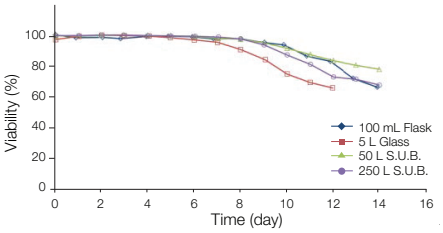

Figure 2. Viability across scales in Dynamis Medium and EfficientFeed C+ Supplement.

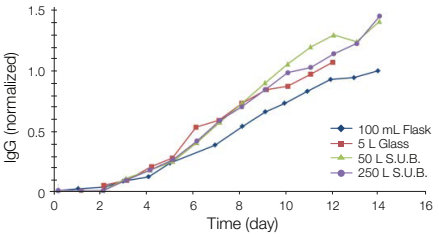

Figure 3. Normalized titer across scales in Dynamis Medium and EfficientFeed C+ Supplement.

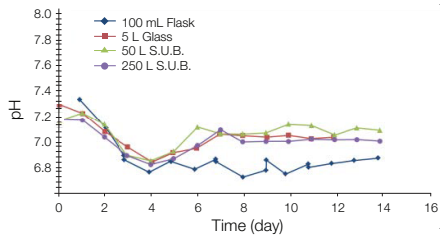

Figure 4. Offline pH across scales in Dynamis Medium and EfficientFeed C+ Supplement.

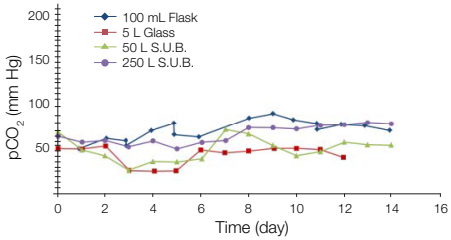

Figure 5. Offline pCO_2 across scales in Dynamis Medium and EfficientFeed C+ Supplement.

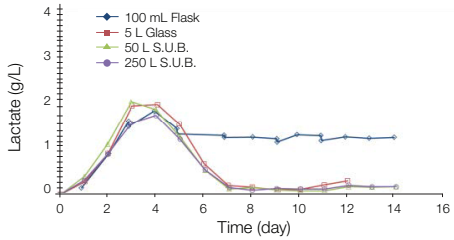

Figure 6. Lactate profile across scales in Dynamis Medium and EfficientFeed C+ Supplement.

Results

Leveraging the continuous feeding and variable CO_2 gassing strategy with Dynamis Medium and EfficientFeed C+ Supplement, process performance was consistent from 5 L to 250 L bioreactors. As shown in Figures 1 and 2, the peak cell concentrations achieved in the bioreactors were 2.5–2.8 x10^7 vc/mL, and cell viability >65% was maintained at harvest. The productivity trends in Figure 3 show that the bioreactors were comparable across scale, and showed a 50% improvement over the performance in the shake flask.

The consistent performance of the fed-batch process upon scale-up in the bioreactors was achieved by the successful implementation of a pH shift. In Figure 4, all bioreactors showed the same trend, with the pH shifting from 7.2 to 6.8 on day 4, then returning back up to 7.0 by day 7. This was in contrast to the shake flask, where pH remained low from day 4, until harvest. While the pCO_2 trends in Figure 5 varied slightly, the buffering capacity of the medium and feed still supported a comparable pH across bioreactor scales. The biggest impact of this feeding and pH strategy is seen in Figure 6, which shows the trends in lactate profile. Across scales, cultures in the bioreactors had the same lactate accumulation trend, with a peak of 2 g/L at day 3 followed by consumption of the lactate. This reutilization of lactate was not seen in the shake flask culture; this is likely attributable to the lower pH and lower titer observed in that culture.

Conclusion

Dynamis Medium with EfficientFeed C+ Supplement supports a scalable pH control strategy in HyPerforma Single-Use Bioreactors. The variable gassing strategy along with the robust medium enables optimal nutrient supply and minimal lactate accumulation, resulting in consistent process performance in bioreactors ranging from 5 L to 250 L in scale.

Find out more at **thermofisher.com/dynamis** and **thermofisher.com/efficientfeedplus**

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