FAST TRACK API MANUFACTURING FROM SHAKE FLASK TO PRODUCTION SCALE USING A 1,000 L SINGLE USE FACILITY

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INTRODUCTION

The application of a slimline scale-up concept based on a highly robust monoclonal antibody CHODG44 platform process dramatically decreases time to market. Further time-saving is possible by consequently using a fully disposable production facility. Here, we present data, that this strategy uncompromisingly results in minimum time expenditure of only 3 months from the Research Cell Bank to the final Drug Substance.

CONCLUSION AND PROSPECTS

With these experiments a fast track concept for the manufacturing of active pharmaceutical ingredients in single-use bioreactors combined with a robust and reliable process could be shown leading to a very short manufacturing timeline from clone to product. An optimally established slimline scale-up strategy applying the calculated specific volumetric power input as the key scale-up parameter was used. Results regarding titer as well as reproducibility in key process parameters were highly compa-rable. We conclude, that the applied scale-up method is feasible for single-use bioreactors in production scale as well as for non single-use bioreactors in laboratory scale. Additionally, the excellent similarity between shake flasks and production scale bioreactors could be the basis for a leaner process development without the necessity for lab scale bioreactor experiments in the future. This might be a significant advantage in competition for pharmaceutical and biotech companies in the admission of new drugs.

EQUIPMENT AND METHODS APPLIED

Determination of Power Input

The unaerated power number (Ne_0) of the stirrer system in the bio-reactors was calculated according to Liepe, 1988. With this correlation Liepe considers the influence of important geometric dimensions of the stirrer to Ne_0.

[1]

$$Ne_0 = 5.9\left(zRb^{0.8} \cdot \frac{hRb}{d_R} \cdot \left(0.6\sin(\alpha)^{2.1}\right)\right)^{0.9}$$

zRb = quantity of stirrer blades, hRb = maximal vertical height of stirrer blades, d_R = diameter of stirrer, α = blade angle.

Thereafter, the specific volumetric power input was calculated according to Eq. (2). The respective dimensions for the diameter of the stirrer (d_R) are as follows: 74 mm RALF+, 203.2 mm 200 L XDR™, 304.8 mm 1,000 L XDR™. The density of the liquid was set at 1.03 kg L^{-1}.

[2]

$$\frac{P_0}{V_L} = \frac{Ne_0 \cdot 1000 \cdot \rho \cdot \left(\frac{n_R}{60}\right)^3 \cdot d_R^5}{V_L}$$

P_0 = power number, VL = liquid volume, ρ = liquid density, n_R = agitation rate.

Expression System, Media and Feeds

In this study a CHO DG44 cell line was used to produce a monoclonal antibody (mAb) in a high titer fed-batch process. Clone and proprietary media composition were provided by the Cellca GmbH (Laupheim, Germany).

Production Equipment - Bioreactors

Cell mass expansion in seed bioreactors was performed in a 50 L XDR™ bioreactor and subsequently in a 200 L XDR™ bioreactor. Scale-up and production were performed in parallel in a 200 L XDR™ bioreactor and in a 1,000 L XDR™ bioreactor (Xcellerex Inc., Marlborough, MA, USA).

Figure 1: The Rentschler 2012 "Facilty of the Year", GMP Facility. 50 L XDR, 200 L XDR and 1,000 L XDR bioreactor.

RESULTS

Cell Growth

Fed batch cultivations in 4 different scales ranging from 125 mL (shake flask) over 5 L (lab scale) and finally 200 L and 1,000 L as production scale were compared. Through all scales, maximum cell concentrations of approx. 20 × 10^6 mL^{-1} were reached. Cell growth and maxi-mum cell concentrations in 125 mL shake flask and 5 L stirred glass bioreactor were nearly congruent and confirmed during scale-up in 200 L and 1,000 L bioreactors. In addition, maximum cell concentration reached in the XDR bioreactors as 30 % higher compared to the shake flask and the 5 L bioreactor.
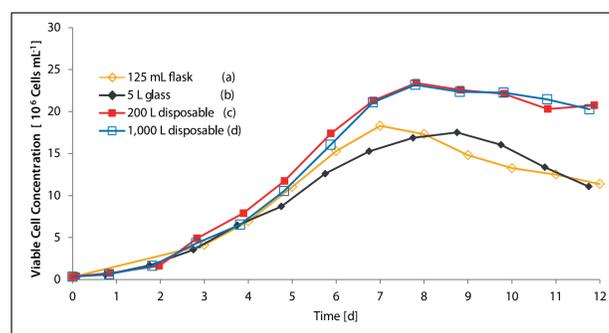

Figure 2: Comparison of viable cell concentration in different fed-batch cultivations in (a) a 125 mL flask, (b) a 5 L glass bioreactor, (c) a 200 L XDR™ bioreactor and (d) a 1,000 L XDR™ bioreactor. Viable cell concentration was measured with a CEDEX (c) and (d) and a CASY (a) and (b). Cultivation conditions were: temperature 36.8°C, pH 7.0, DOT 50 % air saturation, P/V 10-23 W m^{-3}. Cultivation conditions in shake flasks were 7.5 % CO$_2$, 36.8°C and up to 140 rpm shaker frequency in linear shaken modus.

Viability

Progress of the viability in the different scales was nearly identical, as shown in figure 3. Until day 8 of cultivation no significant disparity between all performed runs could be detected. The slight deviation between lab scale runs (shake flask and 5 L bioreactor) and production runs (200 L and 1,000 L bioreactor) were caused by a different sample treatment.
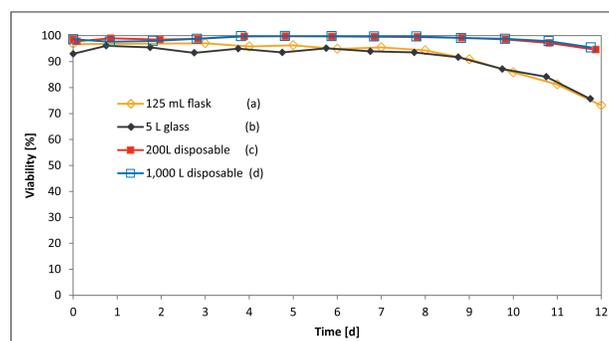

Figure 3: Comparison of viability in different fed batch cultivations in (a) a 125 mL flask, (b) a 5 L glass bioreactor, (c) a 200 L XDR™ bioreactor and (d) a 1,000 L XDR™ bioreactor. Viability was measured with a CEDEX (c) and (d) and a Casy (a) and (b). Cultivation conditions were: temperature 36.8°C, pH 7.0, DOT 50 % air saturation, P/V 10 -23 W m^{-3}. Cultivation conditions in shake flasks were 7.5 % CO2, 36.8°C and up to 140 rpm shaker frequency in linear shaken modus.

Specific Volumetric Power Input

The specific volumetric power input for all stirred bioreactors in the study was maintained constant among the different cultivation sys-tems and during the process phases. In the beginning of the cultivation a value of 10 W m^{-3} was applied through all scales and kept constant until cultivation day 3. During this phase the cultivation volume was also considered to be constant (no feed addition). From cultivation day 4 on, the specific volumetric power input was subsequently increased as a function of the stirrer speed and the liquid volume (see Eq.2). The maximum value in all scales was calculated to be approximately 25 W m^{-3}. During the last days of cultivation the specific volumetric power input slowly dropped to a value of around 20 W m^{-3} due to an increasing ef-fective bioreactor volume caused by feeding.
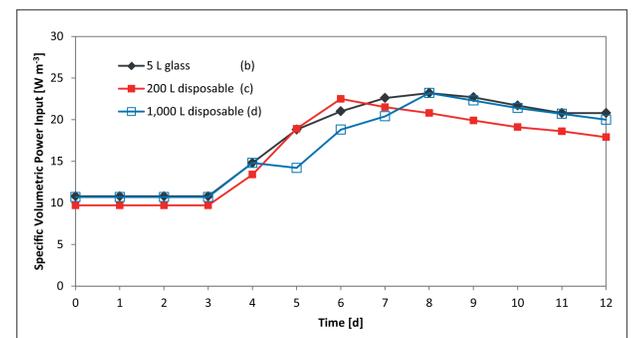

Figure 4: Comparison of specific volumetric power input in different fed-batch cultivations in (b) a 5 L glass bioreactor, (c) a 200 L XDR™ bioreactor and (d) a 1,000 L XDR™ bioreactor. Specific volumetric power input was calculated according to equation (2). Cultivation con-ditions were: temperature 36.8°C, pH 7.0, DOT 50 % air saturation, P/V 10 -23 W m^{-3}.

Product Formation at Different Scales

According to figure 5 all cultivations show a comparable product yield at the different scales. Nonetheless, the product titer achieved in the 5 L glass bioreactor was 30 % higher compared to the shake flask or the 200 L and 1,000 L scale starting from day 6 until the end of the process. Nevertheless, the comparability between 125 mL shake flask and pro-duction scale in XDR™ bioreactors can be considered as excellent.
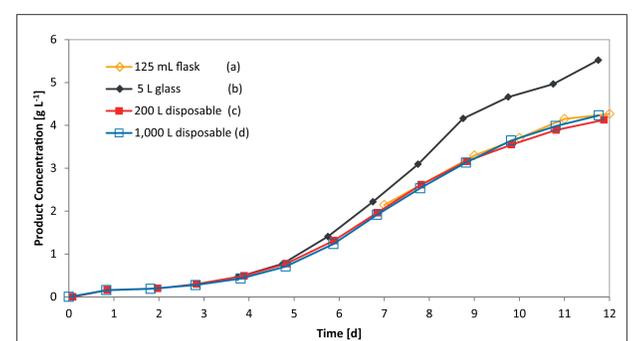

Figure 5: Comparison of product concentration in different fed-batch cultivations in (a) a 125 mL flask from day 7 of cultivation only, (b) a 5 L glass bioreactor, (c) a 200 L XDR™ biore-actor and (d) a 1,000 L XDR™ bioreactor. MAb concentration was measured using a HPLC method; cultivation conditions were: temperature 36.8°C, pH 7.0, DOT 50 % air saturation, P/V 10 - 23 W m^{-3}. Cultivation conditions in shake flasks were 7.5 % CO2, 36.8°C and up to 140 rpm shaker frequency in linear shaken modus.

References

[1] Verfahrenstechnische Berechnungsmethoden, Teil 4 Stoffvereinigen in fluiden Phasen, (Eds; F. Liepe), VCH Verlagsgesellschaft, Weinheim 1988.

[2] Minow B, de Witt H, Knabben I. 2012. Fast track API manufacturing from shake flask to production using a 1,000 L single-use facility, DOI 10.1002/cite.201200136.
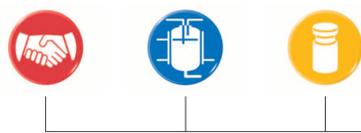
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