Development of a Shaken Scale-Down Model

Characterization of Critical Parameters in Bioprocess Development

© wrw / pixelio.de

A shaken scale-down model at approx. 0.1 L scale for a previously established stirred-tank model at 2 L scale was developed. In the shaken scale-down model online monitoring of pH and dissolved oxygen concentration was performed using the Shake Flask Reader (SFR) by PreSens. Though only a simple, manual control of these two parameters could be realized the shaken scale-down model achieved similar data to the stirred-tank model for viable cell density, specific glycosylation content, and ion exchange chromatography pattern. These results suggest that for this process the importance of pH and DO control might be overestimated and further investigations have to be made for evaluating and comparing the influence of shear stress and power input in both models.
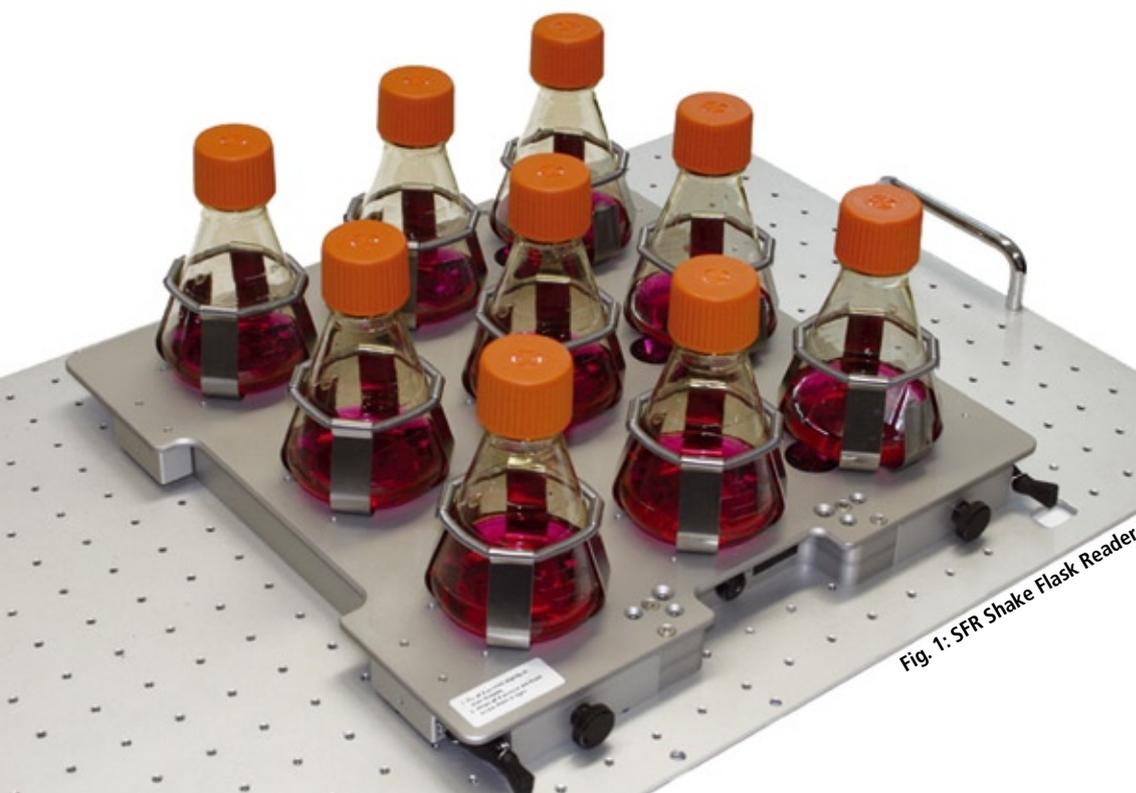
Fig. 1: SFR Shake Flask Reader
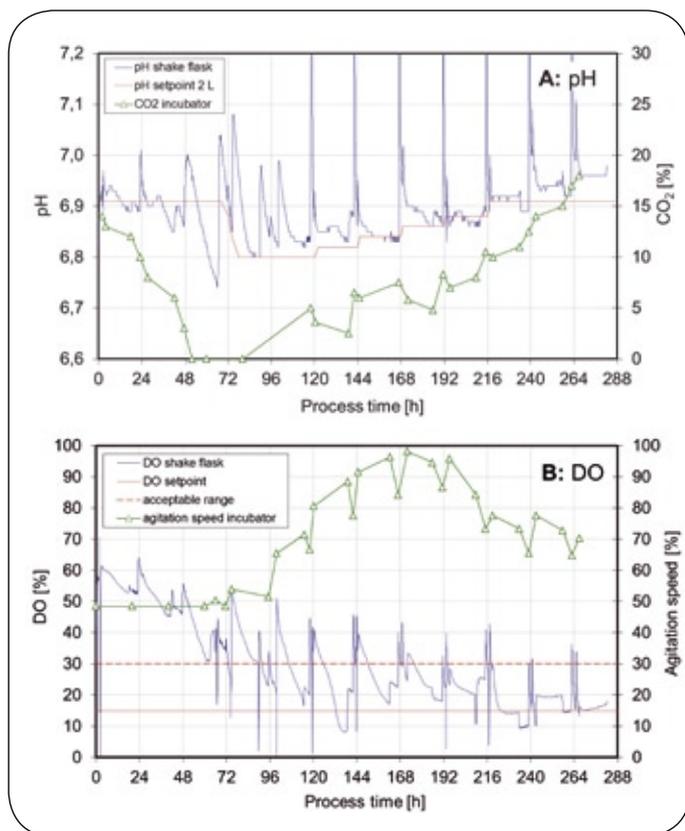
Fig. 2: pH (A) and DO (B) profile at shake flask scale

Scale-down models are used to characterize critical parameters and settings of their proven acceptable range in bioprocess development. This way costs and time can be saved and a reliable scale up can be guaranteed in biotechnical production processes for active pharmaceutical ingredients. An already established 2 L scale-down model for a production process at 1,000 L scale should be further scaled down to a shake flask with approx. 0.1 L working volume. Differences in flow patterns, power input, oxygen transfer, mixing shear stress, foam formation and so on have to be taken into account when trying to establish this shaken scale-down model to a stirred-tank production process. By scaling down the stirred tank process it is possible to increase the number of parallel experiments while reducing the costs. At the same time larger numbers can increase the significance of process characterization studies and a better model quality can be achieved. The system applies chemical optical sensors attached to the bottom of the shake flasks, which are read out non-invasively with the SFR inside the incubator to monitor pH and dissolved oxygen concentration in the shake flasks.

Establishment of a Shaken Scale-Down Model

The SFR was used for online monitoring of the two fundamental process parameters oxygen partial pressure (pO_2) and pH in the shaken scale-down model. In this study a production process for a recombinant monoclonal antibody was performed. Preliminary tests showed that the pH profile might have a significant influence on the performance of this process. As no reliable system for online pH control in shake flasks is available this was realized by controlling the CO_2 content of the inlet gas to the incubator, or by adding base to the medium in case the pH value dropped below the set point. A simplified pH profile was calculated from previous runs of the process at 2 L scale, and applied to reduce the amount of manual interventions at shake flask scale. To assure comparability of the two different scales the pH probe in the 2 L bioreactor and the online chemical optical sensor were tested. A relative constant offset of approx 0.2 pH units was compensated by daily recalibration of both pH measurement devices. Prior to this study an empirical model for oxygen transfer as a function of shaking speed and reaction volume was established on the basis of kLa measurements at shake flask scale. To achieve the set point of 15 % dissolved oxygen (DO) of the 2 L scale the agitation speed of the incubator was adapted manually. An acceptable range
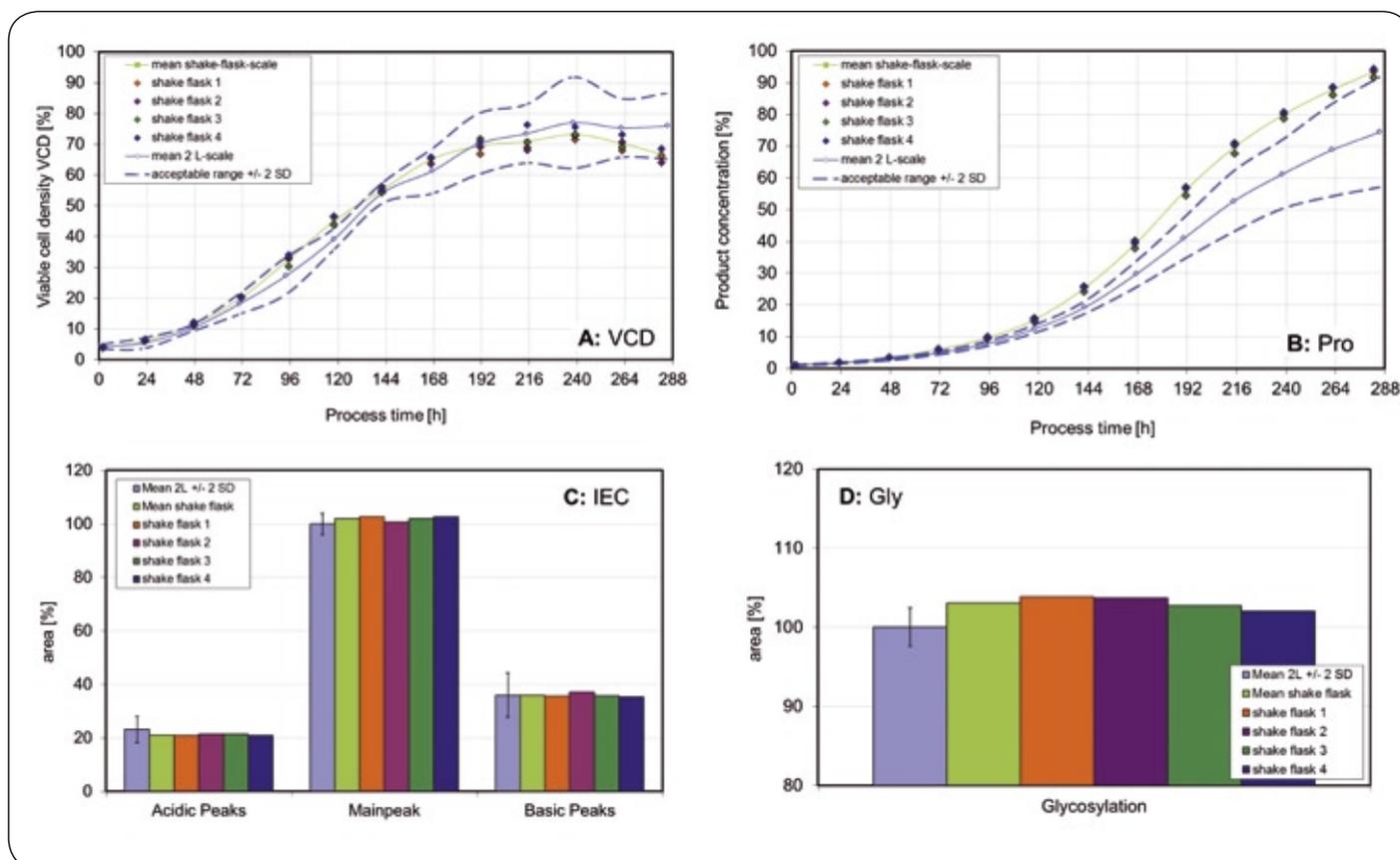

Fig. 3: Process performance at shake flask scale: viable cell density (A), product concentration (B), IEC pattern (C) and glycosylation (D)

between 15 – 30 % DO was aimed at to minimize the number of manual interventions. Further the continuous feed profile of the 2 L scale model was transferred into an intermittent feeding strategy with one bolus feed per day at shake flask scale.

Performance of the Shaken Scale-Down Model

One aim was to realize comparable pH and DO profiles at shake flask scale to assure similar process performance to the 2 L model. The pH in the shake flasks generally lay within a range of 0.05 pH units of the set point during the whole cultivation (see fig. 2 A). Mainly after base and feed addition the pH exceeded its target range, but not for more than 6 hours. Another exception from this range could be measured during the night phase of day 3 when no control was carried out. DO was regulated to a range of 10 – 40 % by only three manual adaptations per day (see fig. 2 B). It exceeded its target range during day 1 – 3 and thereafter daily for 2 – 12 hours. In summary it can be said, that the shake flask scale model failed to realize the preset pH and DO profiles. Quality attributes like viable cell density (VCD), product concentration, peak pattern of ion exchange chromatography (IEC) and relative amount of specifically glycosylated antibody species were also compared to their acceptable range based on values of the 2 L scale model. The scale down model achieved similar process profiles regarding VCD and IEC. The specifically glycosylated antibody percentage exceeded its target range for only 0.7 %, but regarding the accuracy of the measurement technique this is considered uncritical. The product concentration was monitored during cultivation at shake flask scale and reached approx. 20 – 25 % higher values. These results are surprising, considering that the control of pH and DO at shake flask scale was a lot less accurate compared to the 2 L scale model. It might be that the importance of pH and DO control accuracy is overestimated. Control of other process parameters such as power input and shear stress might be a lot more important. Power input in

a shake flask at the used conditions is probably lower than in a 2 L stirred tank bioreactor. The local maximum power input at shake flask scale is much lower, due to the more uniform power input at the flask wall compared to the high shear stress in the vicinity of a stirrer blade in the bioreactor. Oxygen limitation and poor mixing at shake flask scale, which had been reported in literature before, could not be confirmed in these experiments, as similar growth and product quality was reached at both scales.

Conclusion

A 2 L scale stirred-tank model for a production process at 1,000 L scale was further scaled down. The developed shaken scale-down model at approx. 0.1 L scale had very simple, manual control of pH and dissolved oxygen concentration, realized by online monitoring of the two parameters with the SFR Shake Flask Reader. Though this control was of much lower quality compared to a fully online controlled stirred-tank bioreactor, similar data for IEC-patterns, glycosylation content, and viable cell density – important quality attributes – could be achieved with the shaken scale-down model. Other process parameters like power input, shear stress and their influence on process performance in the scale-down model will have to be investigated further.

Authors
Holger Kley, Jan Kreuzmann, Caroline Schuster, and Robert Puskeiler
Roche Diagnostics GmbH, Pharma Biotech Production and Development, Penzberg, Germany

Contact
Presens GmbH
Regensburg, Germany
info@presens.de
www.presens.de