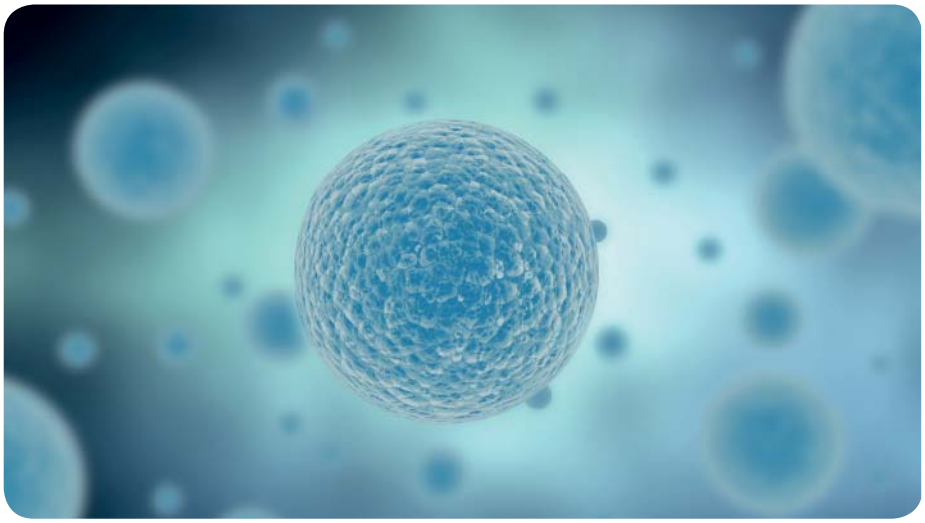
Downscaling in Human Cell Culture

Capabilities of Small Scale Single-Use Bioreactors

In biopharmaceutical applications downscaling of proven processes is a crucial issue while single use technologies gain more and more importance. The following article gives insight to evaluation procedures of a novel, fully instrumented small scale single-use bioreactor.



Increasing process complexity coupled with rising cost pressures and rapidly evolving regulatory requirements makes today's process development efforts a special challenge. The pressure of achieving faster time-to-market for new and innovative biotechnological products has led to the need to optimize every element of the total development workflow. Initial bioprocess development involves cell line optimization, clone selection, and screening for media, feed components and strategies, and other process conditions. Shake flasks, the most common vessels used in early cell and microorganism work, have served the biotechnology industry well over the decades but their limitations for optimizing cell culture or fermentation conditions are well known. Equipment used during screening should mimic the physical and mechanical characteristics of production-scale bioreactors to the greatest degree possible in order to assure, consistency throughout development phases. Ideally, these best practices will support the aims of QbD: that quality measures initiated during development are carried forward and manifested in product quality.

Today's state-of-the-art benchtop systems use sensors and information technology to control, monitor, and record critical process parameters such as temperature, pH, dissolved oxygen, and agitation. As in production scale bioreactors, gassing and feeding proceed according to defined settings. The following application note illustrates how the Dasbox Mini Bioreactor System combined with the new Dasbox single-use vessels supports bioprocess development in human CAP cell culture. Scale-down capabilities were investigated by comparison of 500 ml cultures in a Dasgip Parallel Bioreactor System (PBS) with 170 ml cultures in the Dasbox Mini Bioreactor System using the Dasbox single-use vessel (Dasbox SU). The evaluations were carried out by the company Cevec Pharmaceuticals. The platform expression technologies CAP and CAP-T are based on specific, amniocyte-derived human cell lines and were designed for stable and transient protein production and achieve highest protein yields with authentic human glycosylation patterns.

Fig. 1: A 4-fold Dasbox Mini Bioreactor System with Dasbox single-use vessels

CAP cultivation with PBS

Cevec has established a master cell bank (MCB) of CAP cells growing in suspension, tested and certified according to ICH guidelines and European Pharmacopeia. Simple and reliable protocols allow for the fast generation of customized producer cell lines for pharmaceutically relevant proteins based on the parental permanent cells under controlled

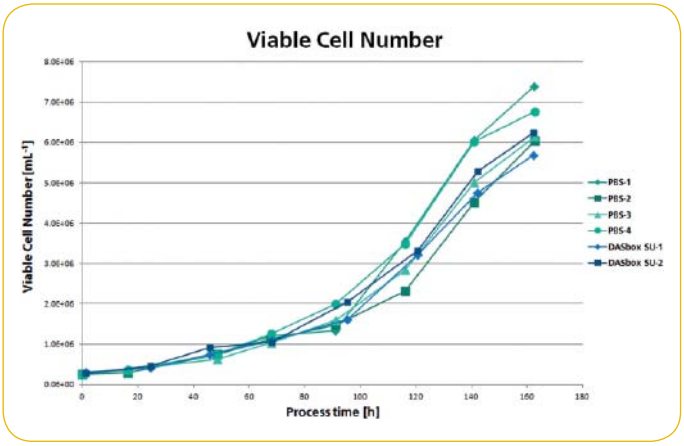


Fig. 2: Viable cell numbers of all experiments with Dasgip PBS and Dasbox SU vessels with average growth rate of 0.02 h^{-1}.

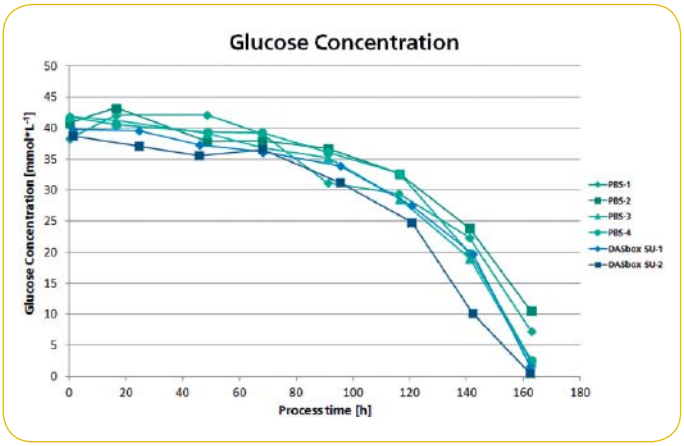


Fig. 3: Comparison of metabolic activity by glucose consumption.

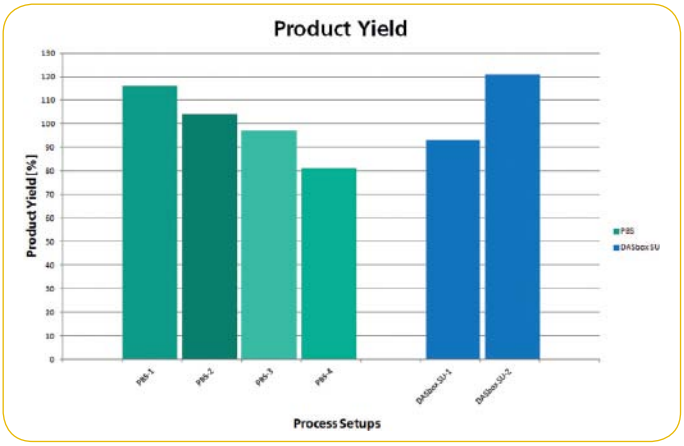


Fig. 4: Product yield normalized to the average protein concentration gained using the PBS.

and optimized conditions. For the required human cell line screening as well as for media optimization, the small working volumes of 100 – 250 ml were used.

Bioprocesses are controlled as precise and effectively as they are in larger scale bioreactors while cell material, media and supplements as well as lab space are saved. Several experiments were carried out aiming at verifying the scale-down capabilities from the PBS to the mini bioreactor system. To overcome the risk of cross-contamination and to reduce time for cleaning, sterilization and assembly they evaluated the novel developed single-use vessel.

Material & Methods

To evaluate the scale-down capability of the new mini bioreactor system and the usability of the new single-use vessel experimental series with two different systems were carried out and compared. A 4-fold PBS for cell culture was used in 500 ml scale experiments. The corresponding small-scale approaches were carried out in a (parallel) Dasbox SU with 170 ml The recombinant human CAP cells producing a pharmaceutically relevant protein were batch cultivated for 7 d (170 h) in Cevec's serum-free, chemically defined CAP medium supplemented with 40 mM glucose and 6 mM glutamine at 37 °C. Initial viable cell density was 3*105 cells/ml. The DO setpoint of 40 % was maintained by a constant stirrer speed and the oxygen concentration in the inlet gas. Stirrer speed was adjusted to 160 rpm (PBS) and 150 rpm (Dasbox SU). The pH value was regulated to 7.1 by addition of 1 M Na_2CO_3 (feeding, speed rate regulated) and CO_2 (submerged gassing). Inlet gas (air, O_2, CO_2 and N_2) was mixed continuously mass flow-controlled. The bioreactors were equipped with pitched blade impellers and liquid-free operated exhaust gas condensors. The precultures were cultivated in 125 ml Erlenmeyer flasks (Corning) with 25 ml working volum using a shaker incubator (37 °C, 5 % CO_2) agitating at 185 rpm (Multitron 2, Infors). The cells were expanded up to a viable cell density of 3*106 cells/ml in the same medium used for bioreactor runs. The critical process parameters were monitored, controlled and visualized online while additionally offline parameters were added manually for collective analysis and storage in a joint database. Daily samples were taken in place. Viable cell numbers, the concentrations of glucose as well as the target protein were determined via semi-automated trypan blue cell counting (Cedex, Roche Innovatis), an automated glucose biosensor (YSI 7100 MBS, YSI Life Sciences) and ELISA, respectively.

Results

The highly comparable results shown in figures 2 and 3 prove the reliability of the process control in both independent experimental series. The viable cell density increases exponentially within all cultivation studies in a reproducible manner with an average growth rate of 0.02 h^{-1}. The corresponding anticyclic glucose consumption thereby illustrates the similar metabolism of the different cultures. Cell viabilities ranged in between 90 – 95 % for each sample. As shown in figure 4 the final product yield reached 80 – 121 % in respect to the average protein concentration gained with the PBS. No differences in cell growth, metabolic activity and protein expression could be observed using the single-use vessels. The results show the successful scale-down from a 500 ml (PBS) to 170 ml (Dasbox SU) bioreactor working volume.

Conclusion

Summarized, the presented results give direct evidence to the scale-down capability of the Dasbox Mini Bioreactor System used with single-use vessels. This proves the system to be a tool for process development with human cell cultures. The small working volumes save material and consumable costs while utilizing single-use vessels drastically reduce turnover-times and thereby labor costs and development times.

References

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