sartorius stedim biotech

CultiFlask 50: a versatile tool for media optimization in biopharmaceutical production

CultiFlask 50 disposable bioreactors from Sartorius offer a fast and economical approach for performing many parallel cell cultivation experiments. Being superior to tissue culture flasks in maximum cell number, medium consumption and ease of handling, they perfectly fill the gap between multiwell plates for initial screening of clones and instrumented laboratory-scale bioreactors for final fine-tuning of process conditions.

Before the final production phase of a biopharmaceutical compound, the process development team has to determine the proper conditions with which to achieve the highest yield in the shortest time possible. Every production cell line as well as every single phase of the manufacturing process has its own distinct requirements in terms of medium composition, additional requirement for growth factors or nutrients, process parameters such as temperature, and other parameters. Thus process development in general, and medium optimization in particular, usually means that many cell culture experiments have to be performed to cover all combinations of the different parameters. For obvious reasons a high degree of parallelism of the respective cell culture vessel design would be desirable: the higher the number of experiments performed in a given period of time, the earlier large-scale production can start.

Instrumented bench-top bioreactors are definitely the method of choice for the last stage of the optimization procedure. By this time most of the crucial parameters have been clarified, and this stage is aimed at assuring smooth transitions from lab- to pilot- to production-scale. For preliminary experiments, in contrast, these systems are much too sophisticated, too expensive and require considerable laboratory resources.

Multiwell plates are exceptionally useful for very large numbers of parallel experiments; thus they are often applied for high-throughput screening applications such as clone screening. But they do not lend themselves easily to the applications outlined above because they are prone to liquid loss by evaporation, and thus do not allow growth of cells for more than a few days. Furthermore, they allow only a very small sample volume, insufficient for subsequent analytical steps.

Joachim Luecke

Sartorius Stedim Biotech GmbH, August-Spindler-Strasse 11, 37079 Goettingen, Germany. Correspondence should be addressed to J.L. (joachim.luecke@sartorius-stedim.com).

Figure 1 | CultiFlask 50 disposable bioreactor.

Tissue-culture flasks allow larger sample volumes, but the cell numbers usually are low due to oxygen constraints; also, the handling of reasonable numbers of tissue culture flasks is labor-intensive and tedious.

The CultiFlask 50 disposable bioreactor (**Fig. 1**) resembles the classic centrifuge tube design. The recommended working volume is 5–30 ml. Handling steps such as medium exchange are performed easily. Its vented screw cap is equipped with a hydrophobic PTFE membrane, which not only serves as a sterile barrier, but also minimizes loss of liquid by evaporation. CultiFlask 50 are operated in an orbital shaker; the shaking mechanism produces excellent oxygen and carbon dioxide exchange rates, while minimizing hydromechanical stress—particularly important when growing cells, which are susceptible to shear forces. Foaming, which often impairs sufficient oxygenation, is negligible. The contamination risk is reduced to a minimum, as there is no stirring mechanism extending into the

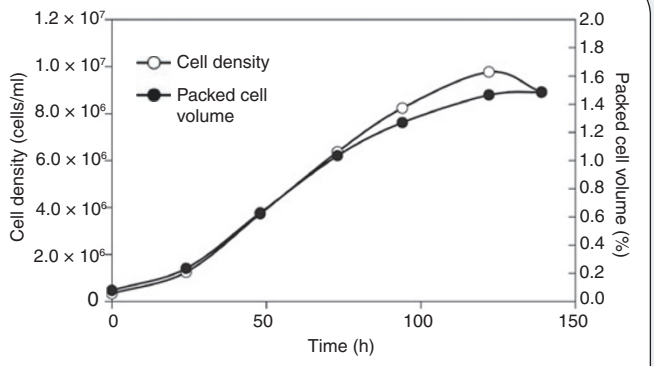

Figure 2 | Cultivation to high cell density. Increase in cell density and packed cell volume of CHO DG44 cells grown for ~5 d in 10 ml of proCHO5, shaken at 180 r.p.m. (37 °C, 5% CO_2).

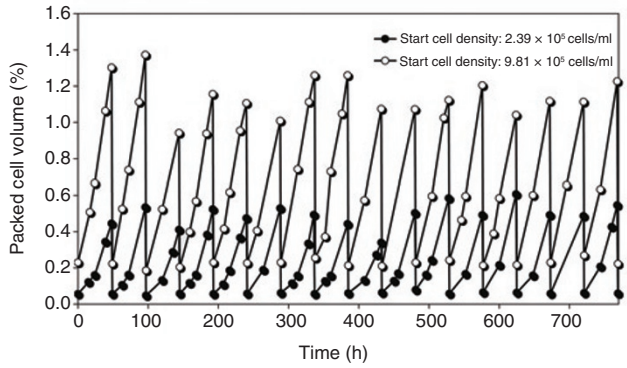

Figure 3 | Long-term cultivation. Increase in cell density and packed cell volume of CHO DG44 cells grown in 10 ml of proCHO5, shaken at 180 r.p.m. (37 °C, 5% CO_2), starting from two different cell densities.

tube. Owing to the presence of a good oxygen supply, cells grow to numbers as high as those in a classic bioreactor; this legitimates the consideration of each tube as a single, non-instrumented bioreactor. Provided there is sufficient incubation shaker space available, hundreds of parallel cell culture experiments are easily performed; labor, equipment and consumable costs are more than reasonable.

Typical cultivation experiments

We performed the first experiment in the CultiFlask 50 disposable bioreactor with CHO DG44 cells in a working volume of 10 ml proCHO5 serum-free medium. The incubation shaker was set to 180 r.p.m. with a shaking amplitude of 50 mm (37 °C, 5% CO_2). Starting with a cell density of 3.37×10^5 cells/ml, a maximum cell density of 9.77×10^6 cells/ml was achieved in approximately 5 d (**Fig. 2**; data expressed as cell/ml as well as percentage packed cell volume (PCV); biomass determination performed with Sartorius VoluPAC™ tubes). These data show that the CultiFlask 50 disposable bioreactor tubes provide cell numbers similar to those achieved in an instrumented bioreactor.

In a second experiment, we grew CHO DG44 cells under the same conditions as in the previous experiment, but with two different seeding densities and over a prolonged period of time (**Fig. 3**). The cultures were run under batch conditions; every second day the cell density was adjusted to the initial inoculation density. A start cell density of 9.81×10^5 cells/ml proved advantageous over the lower seeding density of 2.39×10^5 cells/ml in terms of maximum cell density to be reached. Moreover, these data clearly show that CultiFlask 50 can be used to grow cells under batch conditions for about one month, which makes CultiFlask 50 particularly useful for purposes such as cell or bioreactor monitoring.

Conclusion

CultiFlask 50 disposable bioreactors are versatile tools for process development, optimization and monitoring. They provide gentle mixing of the medium and excellent gas transfer rates, which allows high cell densities to be reached. Therefore, the tubes may justifiably be considered independent, non-instrumented bioreactors that can be applied in large numbers for parallel cell cultivation experiments. The tubes are easy to use and disposable; they do not require a large initial investment nor elaborate training. Because of the high degree of parallelism they speed up considerably the work of process development and thus also the time-to-market of the final product.

ACKNOWLEDGMENTS

The author thanks M. Jordan, M. Stettler and F. Wurm of the Laboratory of Cellular Biotechnology in the School of Life Sciences of the École Polytechnique Fédérale de Lausanne for their contribution to this work.