SCALE-DOWN OF AN ORBITAL SHAKEN BIOREACTOR: HIGH CELL DENSITY CULTIVATION IN PERFUSION MODE AND VIRUS PRODUCTION

Sven Goebel, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany, goebel@mpi-magdeburg.mpg.de
Lars Pelz, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany
Ilona Behrendt, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany
Matthias Proemmel, Ceva Innovation Center, Germany
Jennifer Altomonte, Technische Universität München, Germany
Tibor Anderlei, Adolf Kühner AG, Switzerland
Tim Bürgin, Adolf Kühner AG, Switzerland
Yvonne Genzel, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg
Udo Reichl, Max Planck Institute for Dynamics of Complex Technical Systems & Otto-von-Guericke University, Germany

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Application of single-use bioreactors has been commonly shown for several cell culture-based production systems including commercial vaccine production. Compared to stainless steel bioreactors, competitive cell growth characteristics as well as virus yields can be reached [1]. In addition to conventional stirred tank reactors (STR), wave bioreactors or orbital shaken bioreactors (OSBs) are available that rely on alternative mixing regimes. For small-scale screening of clones and media, cell maintenance and process optimization, OSBs are the most widely used system. Besides their simple design and ease of handling, OSBs allow for robust processes due to reduced mechanical stress caused by stirring and aeration [2]. Furthermore, scale-up ($\leq$ 2500 L) is simplified as larger OSBs rely on the same basic principles for mixing and aeration (e.g. bubble-free surface gassing). Particularly for high cell density (HCD) processes, high oxygen transfer rates, short mixing times, and low shear stress are beneficial. Until now, the step from spin tubes or shake flasks into larger OSBs was rather large, as only the OSB SB10-X (Kühner AG, Switzerland) with a minimum working volume (wv) of 4-5 L was available. In this study, a novel scale-down 3 L vessel module (wv = 1-3 L) for the OSB SB10-X was evaluated for cultivation of suspension BHK-21 cells (CEVA, Germany) in perfusion mode to HCD. Cultivation was carried out in serum-free medium in a 3 L and 10 L single-use standard bag with 3 L and 5 L initial wv and 100 and 70 rpm shaking frequency with a shaking diameter of 50 mm, respectively. For perfusion, an alternating tangential flow system (ATF2, Repligen) with a cut-off of 0.4 µm (SB10-X) and 0.5 µm (SB3-X), respectively, was used. Following an initial batch phase of 2-3 days, perfusion was initiated. After a complete media exchange, cells in the 3 L vessel module were infected with a fusogenic oncolytic virus (rVSV-NDV, recombinant vesicular stomatitis virus-Newcastle disease virus) at a cell concentration of 44.5×10^6 cells/mL at a multiplicity of infection (MOI) of 10^{-4}. The obtained data were compared to a cultivation of BHK-21 cells in the standard SB10-X module (infection at a cell concentration of 12.5×10^6 cells/mL with yellow fever virus WHO 17D-213/77 with an MOI of 10^{-3}) and to a cultivation in a 1 L STR. The novel 3 L vessel module allowed for a successful and direct scale-down utilizing the SB10-X backbone without the need for further optimization. For both the SB10-X and the 3 L vessel module, the ATF system was successfully coupled and cell concentrations of 32.7×10^6 cells/mL and 45.9×10^6 cells/mL were reached with high viabilities above 98%, respectively. A faster doubling time (t_D=22 h) was observed in the 3 L vessel module compared to the SB10-X system (t_D=27 h). For rVSV-NDV production, similar infectious virus titers were reached compared to perfusion cultivations of BHK-21 cells in a 1 L STR. Volumetric media consumption was significantly reduced in the 3 L vessel module, facilitating the implementation of OSB systems in non-industrial research environments. All in all, we demonstrated the adaptability and scalability of the single-use OSB system for the production of various viruses in HCD perfusion mode.

References

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