ORBITAL SHAKEN BIOREACTOR FOR INFLUENZA A VIRUS PRODUCTION IN HIGH CELL DENSITY CULTIVATIONS

Juliana Coronel, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
coronel@mpi-magdeburg.mpg.de
Ilona Behrendt, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
Tim Bürgin, Adolf Kühner AG, Switzerland
Tibor Anderlei, Adolf Kühner AG, Switzerland
Volker Sandig, ProBioGen, Germany
Yvonne Genzel, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
Udo Reichl, Max Planck Institute for Dynamics of Complex Technical Systems, Germany

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The majority of large scale cell culture processes is performed in stirred tank bioreactors (STR) and process development employs scale down models of the same bioreactor type. However, for screening of clones or process conditions at even smaller scale, shake flasks (SF) represent the most widely used model. Occasionally, SF allow for robust processes that cannot be transferred to STR because sensitive cells cannot cope with mechanical stress in STR due to stirring and aeration. Orbital shaken bioreactors (OSB) are a valuable alternative to STR as the transfer from SF to OSB is simplified because the systems rely on the same basic principles for mixing and aeration (e.g., bubble-free surface gassing). In particular, high oxygen transfer rates and short mixing times combined with low shear stress can also be achieved in OSB. These benefits may be even more pronounced in high density culture processes.

In this study, the SB10-X bioreactor (Kühner AG, Switzerland) was evaluated for the production of influenza A virus. Avian AGE1.CR.pIX cells (ProBioGen AG, Germany) were cultivated in the chemically defined medium CD-U3 (Biochrom-Merck, Germany) in 10 L single-use standard bags with 5 L initial working volume (w.v.) and 70 rpm shaking frequency. For perfusion, either an alternating tangential flow system (ATF2, Repligen, 500 kDa cut-off) or a tangential flow filtration unit (TFF, Spectrum Labs, 0.2 µm cut-off) was used (Figure 1). After infection with influenza virus A/PR/8/34 (H1N1) at a MOI of 0.001, the w.v. was increased up to 9 L while perfusion was maintained, similarly to a hybrid perfusion fed-batch strategy previously proposed [1]. In addition, the shaking was increased to 90 rpm during virus production phase.

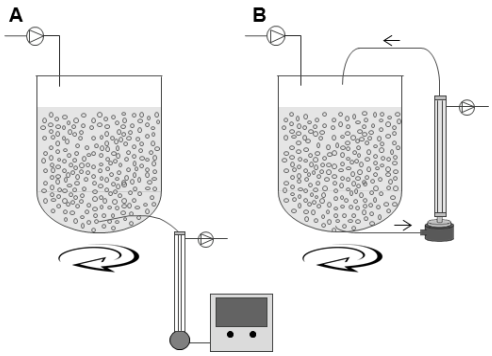

Figure 1. The SB10-X orbital shaken bioreactor was operated in perfusion mode coupled to ATF (A) or TFF (B) with a minimum working volume of 5 L for the 10 L bag. A magnetic drive pump (Levitronix®) was used for recirculation in the TFF.

Both retention devices were successfully coupled to the OSB and concentrations up to 50 x 10^6 cells/mL were obtained, typically with viabilities higher than 90%. We did not observe a decrease of the specific growth rate in the bioreactor system using either perfusion setup. Regarding virus production, we achieved similar or higher HA titers compared to cultivations of AGE1.CR cells in 1 L STR combined with ATF [2]. On average, the cell-specific virus yields (CSVY) obtained in the OSB cultivations were 2.2-fold higher compared to the best ATF cultivations in STR, and a CSVY of 3500 virions/mL was achieved. A comparison between OSB runs in perfusion versus OSB batch revealed a CSVY 2.5-fold higher in perfusion, showing that the typical batch productivity was exceeded by far. On the other hand, infectious virus titers were usually lower in OSB and further experiments are necessary to better understand virus propagation in OSB in case live vaccine production is intended.

To our knowledge, this is the first report on the application of the single-use OSB in perfusion mode and we demonstrate that the technology can be used successfully for virus production.

References

[1] Vázquez, D. et al., Intensification of MVA and influenza virus production through high-cell-density cultivation approaches. Vaccine Technology VI 2016, ECI Symposium Series.
[2] Genzel, Y. et al., High cell density cultivations by alternating tangential flow (ATF) perfusion for influenza A virus production using suspension cells. Vaccine 2014, 32, 2770–2781.