

HOLLOW FIBER-BASED HIGH-CELL-DENSITY AND TWO-STAGE BIOREACTOR CONTINUOUS CULTIVATION: OPTIONS AND LIMITS TOWARDS PROCESS INTENSIFICATION FOR VIRUS PRODUCTION

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Availability of suspension cell lines and culture media for expansion of up to 20×10^6 cells/mL provide perfect starting points to develop process intensification strategies for vaccine production. Modern hollow fiber-based perfusion systems accomplish up to 500×10^6 cells/mL in CHO cell cultivations. Reaching 10 to 20 fold higher cell concentrations, while keeping cell specific virus yields constant, could make processes with very low cell specific virus yields (10-100 viruses/cell) already to feasible processes. Therefore, all possible process strategies using new media, cell lines and reactor equipment need revisiting.

Data obtained from the production of the modified vaccinia Ankara virus strain MVA-CR19 as well as influenza A/PR/8 virus in either hollow fiber-based high-cell-density (HCD) cultivations (using an alternating tangential flow (ATF) perfusion system) or in two-stage bioreactor continuous cultivations of the suspension cell line AGE1.CR.pIX are presented and critically discussed. Options and limits are highlighted to allow an evaluation of both approaches with respect to scale-up and application to other virus-host cell systems.

Both process strategies were successfully scaled-down into shaker flasks allowing parallel experiments.

Accordingly, perfusion and semi-perfusion at a feeding rate of 0.05 nL/cellxd led to concentrations of AGE1.CR.pIX cells above 60×10^6 cells/mL with neither limitation nor overload of nutrients. For infections in 50 mL, a combined strategy comprising an initial fed-batch phase followed by a periodic virus harvest phase resulted in the highest product concentration. Compared to a conventional batch process at 4 to 8×10^6 cell/mL, maximum titer increased more than 10-fold. Additionally, a 3-fold increase in both cell-specific yield (virus/cell) and volumetric productivity (virus/Lxd) could be obtained. The subsequent scale-up into a 1 L bioreactor with ATF perfusion was equally successful and besides allowed re-evaluation of hollow-fiber cut-off.

Alternatively, a small scale semi-continuous two-stage cultivation system (100 mL scale, two shaker flasks) was established as an approximation for a genuine continuous bioreactor set-up (1 L scale, two-stage stirred tank bioreactor). MVA virus production at both scales resulted in stable titers of MVA-CR19 virus (approx. 1×10^8 IU/mL) for over 18 days suggesting an absence of the “von Magnus effect” compared to influenza virus. PCR analysis confirmed stable maintenance of the recombinant transgene in a MVA-CR19.GFP virus. Such a system may be of interest for continuous production of recombinant MVA-based vaccines and gene therapy vectors in the future.

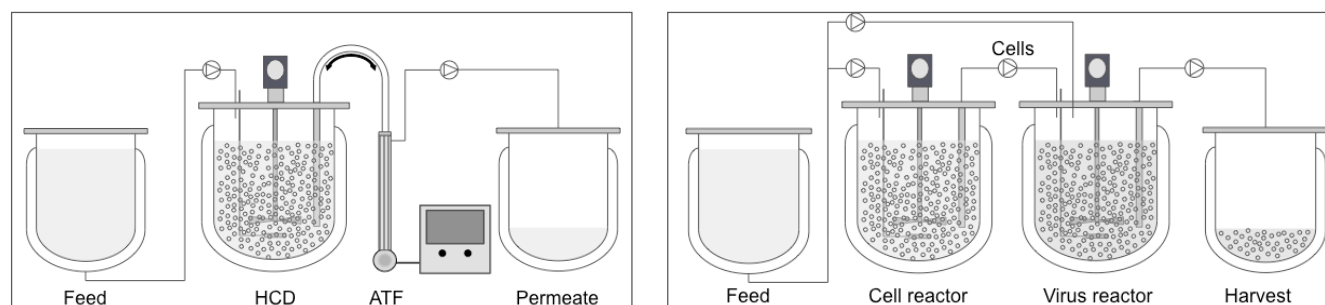


Figure 1: Scheme of a hollow fiber-based HCD and a two-stage bioreactor continuous virus production.

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