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Process optimization for semi-continuous virus production at high cell densities

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PROCESS OPTMIZATION FOR SEMI-CONTINUOUS VIRUS PRODUCTION AT HIGH CELL DENSITIES

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Background. Unlike production of recombinant proteins, continuous production of viral vaccines at high cell densities (HCD) is still constrained by host cell lysis during virus propagation and limited virus recovery from culture broth. Nevertheless, advanced fed-batch [1] and perfusion strategies can be applied to achieve a high-yield virus production processes. In this study, the development of a high-yield semi-continuous process for the production and purification of the modified vaccinia Ankara virus isolate MVA-CR19 and influenza A/PR/8 in HCD cultivations of the suspension cell line AGE1.CR.pIX (ProBioGen AG, Berlin) is presented.

Methods. Depending on the required scale, high cell concentrations (~ 50×10^6 cell mL^{-1}) were achieved either through medium renewal by periodic centrifugation (semi-perfusion) in 50 mL cultivations or using an alternating tangential flow (ATF) perfusion system for 1 L bioreactors. Process development and optimization comprised three phases: 1) assessment of different fed-batch and medium exchange strategies for the propagation of MVA-CR19 or influenza A/PR/8 viruses in 50 mL cultivations; 2) scale-up and process optimization of the high-yield process strategy to a 1 L bioreactor with the ATF system, and 3) integration of a purification process step using magnetic sulfated cellulose particles (MSCP). For both viruses, conventional batch cultivation (no addition/medium exchange after infection) was compared with processes applying fed-batch, periodic medium exchange and the combination of both during virus propagation.

Results. Perfusion and semi-perfusion at a feeding rate of 0.05 nL/cell×d was suitable to propagate AGE1.CR.pIX cells above 60×10^6 cells/mL with neither limitation nor overload of nutrients. For infections in 50 mL, the application of a combined strategy comprising an initial fed-batch phase followed by a periodic virus harvest phase resulted in the highest product yield with a more than 10-fold increase, compared to the conventional batch processes at 4 to 8×10^6 cell/mL [2]. Additionally, a 3-fold increase in both cell-specific yield (virus/cell) and volumetric productivity (virus/L×d) could be obtained. Although product harvesting was suboptimal when up-scaling to a 1 L bioreactor with ATF-system, comparable increases in virus yields and productivity with respect to the conventional batch process were observed. In all cases, cell-specific yields and volumetric productivities reached their peak values at the peak virus concentrations, indicating that the process should be stopped at that time point. Eventually, selection of the optimal pore size of the membrane of the ATF-system allowed semi-continuous harvesting of the produced viruses and its purification with MSCPs with a recovery of about 50%.

Conclusion. Compared to conventional batch processes, the developed HCD process offers significantly higher productivities including the option to integrate a purification step in a semi-continuous mode. Overall, the results show that there is a great potential for semi-continuous HCD processes for the production of viral vaccines in larger scales, which could intensify the discussion towards the establishment of true continuous production process.

References.

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