STIRRED TANKS IN CASCADES AND PLUG-FLOW TUBULAR BIOREACTORS FOR CONTINUOUS PRODUCTION OF VIRAL VACCINES

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Seasonal and emerging viruses are a major threat for human and animal health worldwide. Whole-virus vaccines are currently produced in batch processes that are either egg- or cell culture-based. Therefore, the shift to continuous processing can be a major technological development that can help to reduce the cost of manufacture and increase vaccine accessibility worldwide [1]. Since continuous processes are known to be more efficient than batch at large production volumes, they can be used preferentially for production of highly demanded viral vaccines. One example is the seasonal influenza virus that causes annual epidemics in human populations worldwide and is currently produced in batch mode. Another virus of interest is Modified Vaccinia Ankara (MVA) virus, which can be used for production of recombinant vaccines or viral vectors [2]. Because both are lytic viruses, one approach to produce them is using continuous stirred tank bioreactors (CSTRs) in cascades, where cell propagation and virus replication occurs in separated vessels [3]. Unfortunately, some viruses produce defective interfering particles that then lead to oscillations in virus levels and low overall production yields for the cascade solution [3]. This phenomenon, known as von Magnus effect, can be overcome, if the virus is propagated in a plug-flow tubular bioreactor (PFBR) using a virus stock of defined passage number for the infection. In this work, we describe the establishment of CSTRs in a cascade and a PFBR system for production of MVA and influenza virus, respectively.

A semi-continuous two-stage shaken cultivation system (two 100 mL shaker flasks; SSC) was established as screening tool for influenza and MVA virus propagation before scaling to a “real” cascade of CSTRs (two 1 L stirred tank bioreactors). The MVA virus strains MVA-CR19 and MVA-CR19.GFP were used, and propagated in the duck cell line AGE1.CR.pIX (all three from ProBioGen, Berlin). In addition, a PFBR prototype system was constructed for continuous influenza virus production [4]. The system consisted of a 500 mL stirred tank bioreactor (360 mL working volume) connected to a PFBR (211 mL, silicone-based tube, 105 m) and was operated with a nominal flow rate of 12 mL/h. PCR analysis was used to monitor the stability of MVA and influenza viruses.

The SSC system resulted in stable production of cells, and influenza virus titers that approached the oscillatory behavior observed in previous experiments [3]. Interestingly, MVA virus cultivated in the SSC system did not show oscillations in the virus titer. Subsequently, production of MVA-CR19 was scaled to the cascade of CSTRs and maintained for 18 days in continuous mode, confirming the absence of a von Magnus effect over 18 days for MVA virus. Also, the PFBR system resulted in stable production of cells, and stable influenza virus titers ranging between 1.5 and 2.5 $\log_{10}$(HA Units/100µL) for pIX and MDCK cells, respectively. Therefore, for the first time, the von Magnus effect of influenza virus observed in a CSTR cascade was overcome using a PFBR.

Overall, it was demonstrated that production of MVA and influenza viruses in continuous mode is feasible using either CSTRs in a cascade or a PFBR system, respectively. Both bioreactor systems can be considered as cost-efficient tools for production of viral vaccines in continuous mode.

[1] Hill et al. 2016, Curr. Opin. Biotechnol. 42:67-73.
[2] Jordan et al. 2013, Viruses 5(1):321–39.
[3] Frensing et al. 2013, PLOS ONE 8(9):e72288.
[4] Tapia, Genzel, Reichl 2016, Patent Application, PCT/EP2016/060150.