UPSTREAM PROCESS INTENSIFICATION USING FROZEN HIGH CELL DENSITY INTERMEDIATES

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Typical seed train operations start by thawing of a single vial followed by several expansion steps. Reaching sufficient absolute cell numbers for production bioreactor inoculation is time-consuming and reduces plant flexibility. Besides long ramp up times, open cell culture operations are a major source of process variability. High cell density cryopreservation (HCDC) is a method of freezing cells in bags instead of vials and at higher cell densities. This offers the advantage of decoupling expansion and production: both steps can be separated in space and time. Room classification could be decreased due to fully closed processing and reproducibility increased due to a reduction of manual handling steps. Furthermore, these frozen seed train intermediates allow global distribution from a central expansion facility to decentralized global production facilities. Besides from advantages in production, these HCDC bags can be used in process development to ensure equal starting points in experimental setups.

In this study, we developed a single-use bag assembly that supports closed filling, freezing, thawing, and inoculation. Before using the bag application, relevant parameters for this process from filling to inoculation were evaluated in vials with different cell lines. We found that the DMSO concentration for optimal freezing must not be higher than 7,5%. Furthermore, direct freezing at -80 °C instead of using a controlled rate freezing method is possible. Maximum concentration of DMSO in cell cultures should not be higher than 0,5 % when cryopreserved cells in bags are used for inoculation. For the idea of seed train intensification, we tested increasing freezing cell densities from 10 to 100 million cells/mL showing comparable growth. Functionality test of this HCDC method in comparison to vials was demonstrated in 4,2 L bioreactors simulating a manufacturing process. Applicability of this cryopreservation technology has been demonstrated using different bioreactors, perfusion systems, and various CHO cell lines.