Upstream process intensification using frozen high cell density intermediates

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Introduction

Standard seed train operations start by thawing of a single 1 mL vial with cell densities of 10 x 10^6 VC/mL. For reaching a sufficient absolute cell number for production bioreactor inoculation, several expansion steps, starting with shake flasks, need to be performed. This leads to long campaign timelines and low plant flexibility. Furthermore, open cell culture operations result in high room classifications during the whole process and are a major source of process variability.

High cell density cryopreservation (HCDC) is a method where cells can be frozen in larger volumes and higher cell densities than today's standard processes due to the use of cryobags. The result is the reduction of the time and manufacturing space required for standard cell expansion process (Fig. 1).

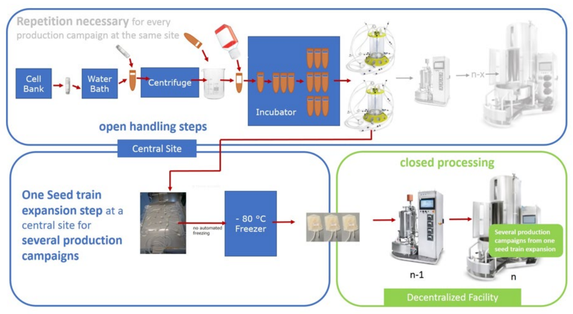

Figure 1: Comparison of a standard versus HCDC production process. *For a standard production process, thawing of one vial per production cycle is necessary. The complete seed train expansion needs to be performed including all open handling steps. With the HCDC application, the seed train expansion needs to be performed only once for several production campaigns. Additionally, the final production step does not need to be performed at the same facility, thus can be decoupled in space and time.*

A lower room classification of the cell culture area in GMP manufacturing could be considered due to the reduced number of manual handling steps before the main stage bioreactor (closed processing). In addition, the HCDC intermediates allow global distribution from a central expansion facility to decentralized global production facilities. R&D and process development teams will also benefit from utilizing HCDC intermediate bags to create equal starting points for reduced variability.

For the development of the HCDC process, cellular sensitivities needed to be determined to identify critical process steps. Standard protocols demand freezing rates of about -1 to -2 °C/min to prevent cell damage. This is hard to attain with bags. To evaluate if there is a negative impact with uncontrolled freezing, vials with a VCD of 10 x 10^6 VC/mL were frozen using 3 different techniques: either frozen with a controlled rate freezer, with a Cool Cell Box, or stored directly at -80 °C. Vials were thawed in a water bath at room temperature and used for the inoculation of 30 mL bioreactor tubes (n = 5) with a starting cell density of 0.3 x 10^6 VC/mL.

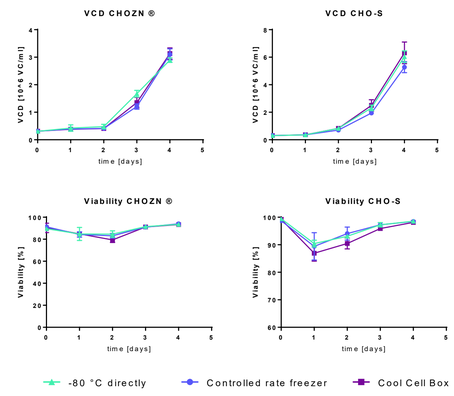

Figure 2: Comparison of different freezing methods. *Cellular sensitivities were determined by using different freezing techniques and monitoring growth and viability after thaw.*

Growth and viability were monitored daily over 5 days and was comparable, leading to the assumption that controlled rate freezing is not necessary for bags (Fig. 2).

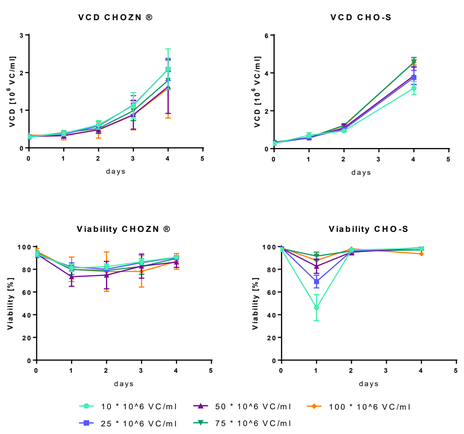

Figure 3: Effect of different cell densities during the freezing process. *Cells were frozen with different cell densities. Growth and viability were monitored after the thaw.*

Conceptually, the advantages of HCDC would be highest with very high cell densities. Based on this, cells were frozen with different cell densities from 10 x 10^6 VC/mL (control) up to 100 x 10^6 VC/mL. Vials were thawed in a water bath at room temperature as previously described, used for inoculation of 30 mL bioreactor tubes, and growth and viability were monitored daily for 5 days. No systematic difference could be seen among the conditions showing that freezing with higher cell densities does not have a negative impact on growth (Fig. 3).

For the bag filling process, a bag assembly prototype was designed (Fig 4). The bag assembly consists of ten 250 mL bags for making HD cryopreserved cell bags, one waste bag, a connection tube for prefilling of the cryo medium as well as a connecting tube for a bioreactor.

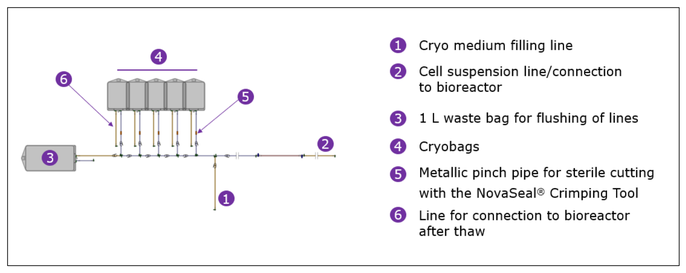

Figure 4: Bag Assembly prototype.

For filling, 50 mL cryo medium with a concentrated DMSO-concentration of 22.5 % need to be prefilled in the bags. Subsequently, bags are filled with cell suspension to the desired volume and disconnected with the NovaSeal™ crimping tool and stored at -80 °C.

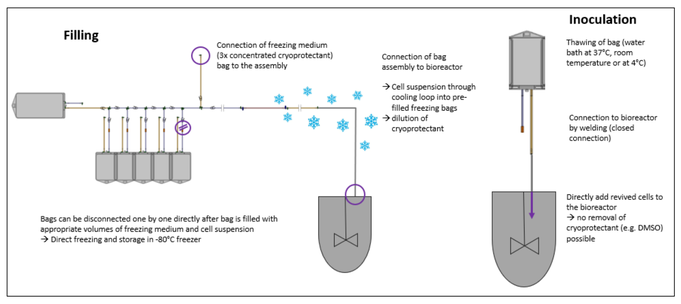

Figure 5: Simplified HCDC Process. *Freezing medium with a concentrated DMSO concentration is prefilled in the cryo bags. The bag assembly is connected in a closed processing manner to the bioreactor and cell suspension is added through a cooling loop to the freezing medium. Subsequently, the cryo bags can be disconnected one by one directly after filling.*

Proof of Concept

HCDC bags and control vials were created by using an STR perfusion bioreactor or a rocker bioreactor (data not shown). Subsequently, the HCDC bags as well as the vial controls, which were taken from the STR perfusion bioreactor run between day (D) 6 and 12, were thawed in a water bath at room temperature and used for the inoculation of 30 mL bioreactor tubes (n = 5) with a starting cell density of 0.3 x 10^6 VC/mL.

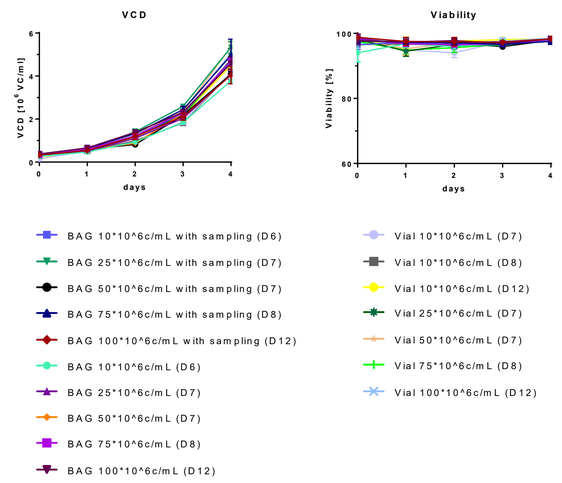

Figure 6: Confirming of cryopreservation process in 30 mL bioreactor tubes*. The prepared bags and vial controls, which were taken from the ATF perfusion bioreactor run between day 6 and 12, were thawed and used for the inoculation of a batch. Growth and viability were monitored daily over 5 days.*

Growth and viability were monitored daily over 5 days (Fig. 6). Both were comparable between bags and vial controls for all cell densities confirming that the process can be performed with cell densities up to 1 x 10^8 VC/mL using the HCDC bag assembly.

Confirmation of the HCDC application

For confirmation of the process, a bag prepared from an ATF perfusion bioreactor was used to simulate the inoculation of an N-1 perfusion bioreactor in a production campaign using a bioreactor with a working volume of 4.2 L as N-1 bioreactor. This bioreactor was inoculated with 0.5 x 10^6 VC/mL using a HCDC bag and operated first in batch mode, changing to perfusion on day 3. On day 7, cell culture broth was bled until a VCD of 0.5 x 10^6 VC/mL was reached again to simulate the inoculation of a final production reactor. The simulated production reactor was also run in batch mode again for the first 3 days and subsequently operated in perfusion. A simulated N-1 bioreactor inoculated with a normal seed train expansion using a vial and subsequently was used as a control. VCD, viability and antibody concentration were monitored daily.

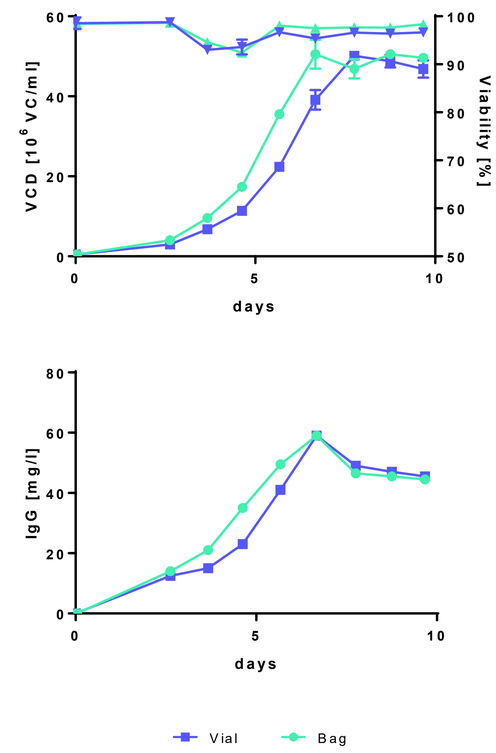

Figure 7: Confirmation bioreactor run*. A bioreactor was inoculated with cells frozen in a HCDC bag. A bioreactor inoculated with a standard vial expansion was used as control. Both were run in batch mode first, changing to perfusion 72 hours later, simulating a perfused N-1 bioreactor in a production campaign (data not shown). Subsequently, both bioreactors were bled to the initial inoculation cell density simulating the inoculation of a production bioreactor.*

After inoculation of both bioreactors with either a seed train originating from a vial or a HCDC bag, growth and product formation was comparable to the simulated productions bioreactors showing that the simulated production stage and the HCDC process could be established successfully.

Conclusion and Outlook

The HCDC process could be established successfully:

Performance was comparable to process with standard seed train expansion.