Introduction to USP – Seed Train Development

Your Objectives:

At the end of the lesson, you should be able to sequence the steps in seed train development

A **seed train** serves to generate an adequate _____ of cells for the inoculation of a production bioreactor. From volumes used for either cell thawing or cell line _____, the cell number will have to be increased, rendering it both time and cost intensive.

A typical cell _____ process begins with the thawing of a cryopreserved cell-bank vial, followed by successive expansions into larger culture vessels, such as shake flasks, spinners, Wave bags, and stirred bioreactors.

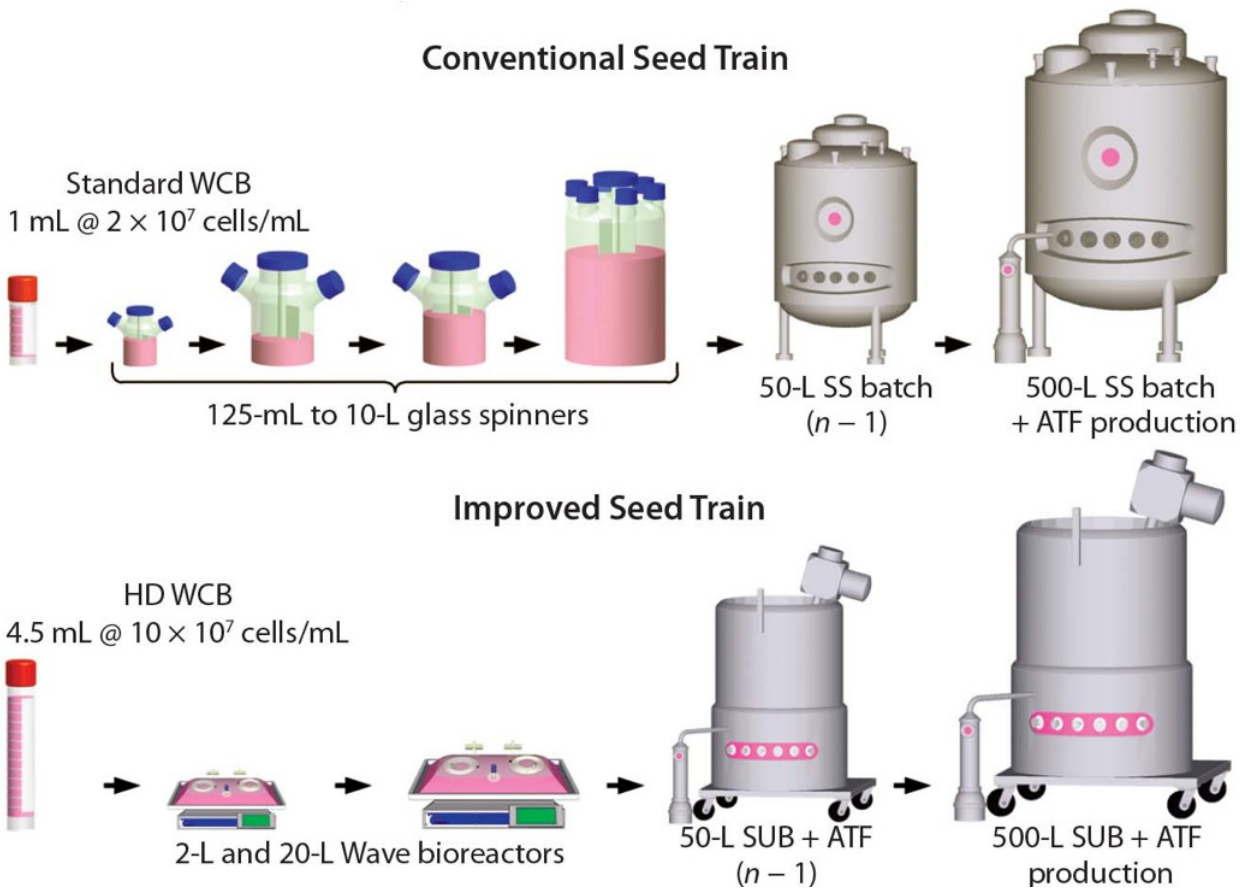
This approach presents several _____: Shake flasks or spinners used in the initial stages require _____ manipulations inside a laminar flow hood, making them vulnerable to contamination and operator error. In addition, since a conventional cryopreserved cell-bank vial typically contains low cell numbers, the seed-train process is time _____. It takes longer and becomes even more complex with much larger production _____ because additional large-volume culture _____ are required to support scale up to a production bioreactor. Finally, production bioreactors being usually inoculated at a density of <0.5 × 106 viable cells/mL also means a necessary growth phase of 5 to 10 days, which is basically unfavourable since, during that period, cell _____ is rather low (as is the initial product yield).

Previous studies have shown that high-density (HD) cell banking can be [ ] at reducing the number of seed-train steps required whilst [ ] operational success in seed-train processes, namely using HD cell banks containing 450 million viable cells/vial to directly inoculate a 20-L Wave bag. One particular [ ] revealed that the use of HD cell-banks would eliminate several intermediate shake-flask expansion [ ] thereby reducing process times by up to nine (9) days.

Another study combined HD cell banks with a single-seed [ ] capable of operating at several working volumes, thus reducing seed-train expansion duration by as much as 60 to 70 percent.**Single-use technology** actually offers numerous [ ] over stainless-steel systems, including enhanced facility flexibility, time [ ] in set-up, cleaning, and [ ], not to mention a reduction in capital investment. Disposable Wave bioreactors are, therefore, routinely used in seed-train expansion processes because they also decrease [ ] risk (typically requiring no manipulations in laminar-flow hoods). Because such bioreactors are disposable, no extra cumbersome and costly [ ] are needed for assembly, cleaning or sterilization.

Perfusion cell-culture processes is in greater demand in this industry as perfusion bioreactors have shown success in manufacturing viable commercial [ ] .

Perfusion [ ] can reach high cell densities in relatively small bioreactor volumes and will remove [ ] product from cultures more quickly than the more conventional batch and fed-batch processes. Because of the aforementioned advantages (and others), perfusion has become the preferred production [ ] over seed expansion.



The illustration compares a novel seed-train process using HD cell banking, disposable Wave bioreactors, and Refine Technology's Alternating Tangential Flow (ATF) perfusion cell-culture technologies over a conventional one. An example of this process for a 500-L production perfusion bioreactor with a conventional seed-train process.

Using the HD cell bank approach involves eliminating two intermediate spinner steps, by allowing for direct inoculation into a 2-L Wave bioreactor. Replacing spinners (125 mL to 10 L) with Wave bioreactors (2 L and 20 L) means significantly reducing the number of required manipulations in a **laminar flow cabinet** (**tissue culture hood**)*, improving operational success by allowing operation under a "closed" system. Additionally, using ATF perfusion technology at the n – 1 stage (50-L bioreactor with ATF perfusion device) enables cell densities to be ≥50 × 106 viable cells/mL, allowing for an inoculation density of 5 × 106 viable cells/mL in the 500-L production bioreactor rather than 0.5 × 106 viable cells/mL in the conventional cell culture process. Higher seeding density helps reduce the 500-L production bioreactor growth phase by approximately five days, saving manufacturing plant time for a 500-L bioreactor operating for 50 days.

*A **laminar flow cabinet**, or **tissue culture hood**, is a carefully enclosed bench designed to prevent contamination of semiconductor wafers, biological samples, or any particle sensitive [ ] .

The Bioreactor Train

Biogen bioreactors are organized into [ ] , each being larger than the next. A bioreactor trains consists of different volumes:

- 60 litre
- 100 litre
- 235 litre
- 750 litre
- 950 litre
- 2000 litre
- 3750 litre
- 15000 litre
- 18500 litre

Each bioreactor feeds into the next. The train is a continuation of the scale up process that begins with the inoculum preparation stage and ends usually with the harvesting process in the largest bioreactor.

Aufgabe Lückentext:

Folgende Wörter bitte in den Lückentext einfüllen.
Jedes Wort kommt einmal vor.
Bitte Gross- und Kleinbuchstaben beachten.

advantages, bioreactors, bioreactor, culture, challenges, contamination, concentrations, consuming, effective, improving, maintenance, manual, materials, method, number, processes, study, steps, savings, sterilization, resources, therapeutics, trains, unstable, vessels