Perfusion: process selection

Introduction

Perfusion is a cell culture process in which cells are retained in the bioreactor with a continuous exchange of medium. This process removes cell waste and spent medium while constantly replenishing nutrients and carbon sources with fresh medium. In this white paper, we will cover four suspension cell culture perfusion types and their typical applications: N-1 and other intensified seed train applications, concentrated fed-batch, intensified fed-batch, and continuous perfusion.

N-1 (intensified seed train)

N-1 and other intensified seed train applications maintain logarithmic cell growth into very high cell densities. One application of intensified seed train perfusion is to generate a high cell density freeze. The high cell density freeze allows seeding directly into several liters of volume and skips smaller flask expansions. From there, N-1 perfusion can be used again to reach very high cell densities, while maintaining logarithmic growth in a smaller seed train reactor.

The increased cell density can be used to bypass additional seed reactors, and potentially seed at a much higher cell density to accelerate production reactor time. Whereas N-1 focuses on achieving high viable cell density at logarithmic growth, the following three perfusion methods are oriented to maximize titer.

Concentrated fed-batch

Concentrated fed-batch is a shorter perfusion run, typically 12–18 days, in which the product and the cells are retained inside the reactor (Figure 1). This perfusion run effectively makes this type of perfusion production a batch-type operation, because the harvest is completed as a single action at the end of the run.

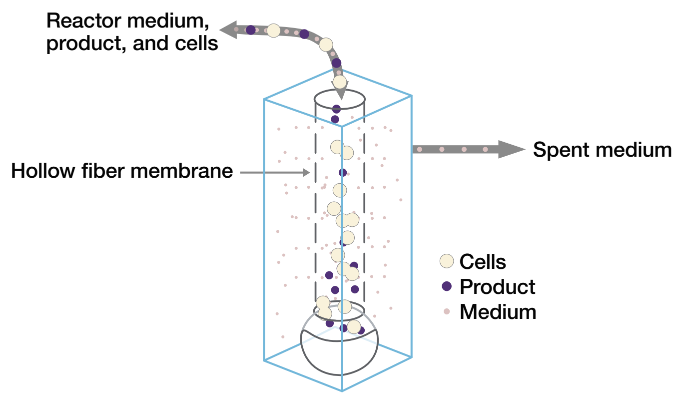

Figure 1. Concentrated fed-batch using alternating tangential filtration. Flow paths are simplified to focus on the separating mechanism and may not represent actual implementation.

In order to achieve retention of product inside the reactor, an alternating tangential filtration or a tangential flow filtration cell retention mechanism must be used. The pores are sized small enough to retain both cells and product, which increase greatly in concentration during the perfusion run. Due to the heavy concentration of product inside the reactor, this application is particularly well-suited to a low-producing cell line that would normally require a concentrating step downstream and prior to separations. This process is also well-suited for stable products since the product is being retained in the reactor the entire run.

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Intensified fed-batch

Intensified fed-batch is a shorter-duration perfusion run, generally 14–20 days. Unlike concentrated fed-batch, product is constantly removed with the medium. Thus, operating an intensified fed-batch process and using a filter-based cell retention mechanism, the filter requires a pore size big enough for product to pass through into the spent medium (Figure 2). The intensified fed-batch application is well-suited for executing perfusion with a cell line that has poor production stability. The application is also usable with toxic or unstable products that need to be continuously removed from the vessel.

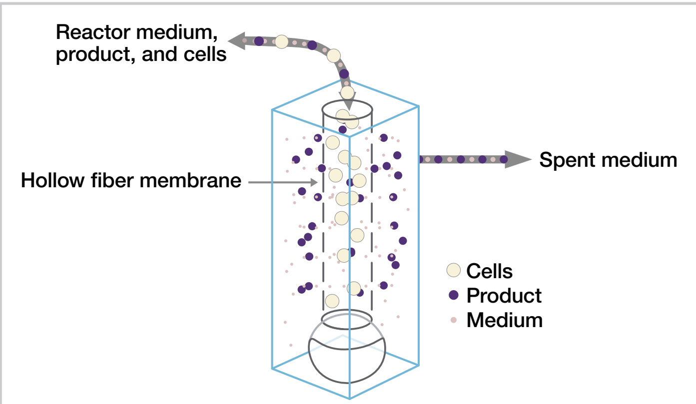

Figure 2. Intensified fed-batch using alternating tangential filtration. Flow paths are simplified to focus on the separating mechanism and may not represent actual implementation.

Continuous perfusion

Continuous perfusion is generally a long process, 30–90 days, and is oriented around sustaining a constant viable cell mass with consistent product output. The application is similar to intensified fed-batch in that product is continuously removed from the reactor, making it suitable for toxic and unstable product production.

A critical requirement for continuous perfusion is that the cell line exhibits relatively stable productivity after selective pressure is removed. Otherwise, productivity may diminish to impractical levels over a long continuous perfusion run. Due to the long operating duration and ability to maintain a steady state, continuous perfusion is a great way to maximize automation benefits and generate a consistent quality profile with minimal process footprint.

All of the perfusion operating approaches discussed are designed to improve process efficiency. A key factor to delivering on process efficiency is to use a medium that allows for lower medium exchange rates, thus making the process easier to manage and realizing increased benefits. Given these needs, Gibco™ High-Intensity Perfusion CHO Medium is an ideal choice for perfusion operations. It allows for high cell densities at low medium exchange rates and comes in Gibco™ Advanced Granulation Technology™ (AGT™) format to facilitate ease of use, especially when working with larger volumes of medium.

Contact your sales representative or get more information at **thermofisher.com/perfusion**

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