Technical Note:

Challenges in Scaling a Microcarrier Based Cell Culture from Spinner Flasks to Stirred Tank Bioreactors.

Introduction

Microcarriers are widely used in cell culture applications to allow anchorage dependent cells to be used in high cell density applications in biologics manufacturing, cell therapy, bioprocessing and research and development, specifically where the target molecule cannot be efficiently expressed using suspension cell culture techniques. Microcarriers have a significantly larger surface area to volume ratio in comparison to monolayer cell culture systems such as T-flasks, cell factories etc.. They are physically robust and allow the use of stirred vessel bioreactors to produce large volumes of the target molecule within a relatively small footprint.

Scaling up a microcarrier based process from a T-75 flask through spinner flask to bioreactor typically utilizes a three-step progression. The first two steps are usually performed in spinner flasks at smaller and then increasingly at larger scale prior to the transfer to a stirred tank bioreactor.

Step 1. Microcarriers are screened to identify the most suitable microcarriers for the specific cell type and the projected density being cultured. Selection includes material type, sphere size, surface area and whether the microcarrier surface has a surface charge or not.
Step 2. Critical process parameters are identified and optimized, the most critical of which are the selection of the optimal culture media, identification of the correct conditions under which maximum microcarrier pairing with the cells being cultured is obtained, and determination of the appropriate detachment method to remove the cells from the microcarrier.
Step 3. Transfer of the process from spinner flasks to stirred tank bioreactors. This is a critical part of the scale up process because the methods of operation and the hydrodynamic operations of stirred cells and bioreactors are very different and can significantly impact cell growth and therefore final product yield.

There are 5 main differences between spinner flasks and bioreactors that need to be considered when designing the transfer process and adapting the spinner flask SOP to use in a stirred tank bioreactor.

Hydrodynamic Differences and the Impact on Mixing Profiles.

Spinner flasks typically have impellers that are relatively large in proportion to the volume of media in the spinner flask and these impellers typically operate at low revolutions per minute (rpm). Bioreactors, in contrast, have smaller impellers relative to the media volume, the impellers have a different shape, sometimes several impellers are positioned on the same shaft, and they operate at higher rpms to provide efficient mixing. These differences can produce very different mixing profiles which need to be addressed during the scale up process into the stirred tank system, the main areas of variability are in mixing efficiency, and time to reach a homogeneous state.

A cell line that is weakly paired to the microcarrier substrate, and grows well in a spinner flask environment, may be subject to physical detachment in the more aggressive and turbulent conditions inside of a stirred tank bioreactor. Optimization of the cell and microcarrier pairing is therefore essential to allow development of a robust process.

It has been stated (1) that the hydrodynamic properties of spinner flasks used in microcarrier based cell culture cannot be exactly reproduced at scale with a stirred tank bioreactor. However, the same authors also recommended using high mixing speeds, >120rpm at a 250mL spinner flask, to mimic scaling up effects of the higher mixing rpm's typically found in larger bioreactor systems.

Seeding

Some microcarrier-cell combinations can be transferred from spinner flasks to stirred-tank bioreactors, even though they are not as strongly paired to the microcarriers as other cell lines and display weaker cell attachment. Seeding efficiency can be improved for difficult pairings by manipulation of the microcarrier to media ratio, or by adjusting the mixing speed and mixing pattern during the cell pairing process.

Manipulation of the microcarrier/media ratio. Increasing the microcarrier density statistically increases cell-to-microcarrier contact (2) in addition a reduced media volume increases oxygen transfer by increasing the surface area to volume ratio, this effect is complemented by increased surface agitation from the impeller. Improved oxygen availability is crucial for an intensified processes where oxygen consumption is high. Stirred vessel systems may require sparging without this strategy, with aeration kept at a minimum during seeding to prevent cell damage. Post cell attachment, media volume is adjusted to the desired process volume.

Continuous stirring. Continuous stirring is preferred to facilitate even cell distribution on microcarriers (3) especially for consistent, repeatable results. However, continuous shear forces generated by the mixing process can reduce seeding efficiency, this can be counteracted by using a higher initial cell number. However, for weak cell-microcarrier pairings, continuous stirring may be the wrong solution.

Intermittent stirring. For shear sensitive cells, or for cells with weak microcarrier pairings, intermittent stirring may be both a more appropriate process and produce a higher yield. A typical recommended intermittent stirring protocol will be active mixing for 5 minutes followed by no mixing for 30 minutes, up to a total of 6 hours. Theoretically this allows cells to attach to the microcarriers without experiencing constant shear stresses, potentially improving seeding efficiency (4). However, because of the reduced stirring time, this approach can lead to poorer cell distribution and increased cell aggregation onset early in the bioprocess, requiring optimization.

Mass Transfer

The mechanisms of aeration are also very different between spinner flasks and bioreactors, which will impact the rate of oxygen adsorption into the media. Spinner flasks rely on surface aeration where the mass transfer area is the reciprocal of the depth of the liquid media in the spinner flask. For example, a 500mL capacity spinner flask, with vertical side walls, would have the same mass transfer area, determined by the diameter of the vessel, whether the vessel has 100mL, 200mL or 300mL of media in it. That same mass transfer area would in these situations be providing gas exchange to different volumes of culture media. For these reasons, Bellco recommends both a minimum and a maximum working volume for each size of spinner flask to ensure maximum aeration across the media surface, and optimal cell growth conditions see below.

Recommended working volumes. The total culture volume in a spinner flask should not exceed one half of the indicated volume of the flask for proper aeration.

- 100mL flask. Minimum working volume 30mL. Maximum working volume 50mL.
- 250mL flask. Minimum working volume 80mL. Maximum working volume125mL
- 500mL flask. Minimum working volume 200mL. Maximum working volume 250mL
- 1000mL flask. Minimum working volume 300mL. Maximum working volume 500mL
- 3000mL flask. Minimum working volume 900mL Maximum working volume 1500mL

Culture initiation. Initiating a culture in spinner flasks larger than 500 mL is not recommended. Bellco recommends starting from smaller spinner flasks and gradually adapting the culture to larger size flasks.

- **Note:** The impeller speed may need to be optimized when moving to different size flask(s).

Spinner flasks have been shown to produce a uniform dissolved oxygen curves at all points within the media column at a given speed (1), although the curves measured are different at different mixing speeds. This means that the liquid phase mixing is faster than the gas to liquid mass transfer at the liquid/gas interface. This limits the applications of spinner flasks at larger volumes because the mass transfer rate would be insufficient to support either high cell densities or cells with high oxygen demand requirements. Using spinner flasks for these applications requires active sparging of air into the spinner flask to overcome the mass transfer limitations of surface aeration only, this in turn requires the use of a narrower diameter impeller to allow a sparger to be located against either the bottom side wall, or at the bottom of the spinner flask (5).

When microcarriers are added to a spinner flask, the apparent viscosity of the solution increases, which in turn affects the mass transfer balance of the system. For a specified system volume (microcarriers + liquid media) as the solid loading (microcarriers) increases, the amount of liquid media decreases by a corresponding amount. As dissolved oxygen only exists in the liquid phase, as the liquid volume decreases, the amount of dissolved oxygen available for cellular respiration also decreases, eventually to a point where it negatively impacts the output of the system.

Once the optimal mass balance has been determined for the spinner flask process, it will need to be recalculated when the process is transferred to a stirred tank bioreactor. Stirred tank bioreactors are actively sparged with sterile air or oxygen forced under pressure through one or more air diffusers. The mass transfer area is therefore determined not by the surface area of the liquid/gas interface at the top of the liquid media column, but by the size of the bubbles generated by the sparger, and the volumetric gas fraction (gas holdup volume) of the media.

Due to the differences in formats and operation of the two systems, the differences between the mass transfer areas of a spinner flask and a stirred tank bioreactor are in the order of 10X in favor of the stirred tank bioreactor. These have been quoted in the region of $20m^2/m^3$ for a spinner flask and $210m^2/m^3$ for a stirred tank bioreactor (1).

Under optimal conditions spinner flasks when used with microcarriers (4) have been shown to achieve cell densities close to those obtained using larger stirred tank bioreactors.

- Spinner flask, with microcarriers (surface area of $380cm^2/g$) = cell density of $1.7 \times 10^6/ml^{-1}$
- Stirred tank bioreactor, with microcarriers, = cell density of $2.0 \times 10^6/ml^{-1}$

One approach to eliminating as many variables as possible to the scale up and the tech transfer process could be the following step wise process.

a. Start with small spinner flasks (100 – 500mL), operating at higher revolutions per minute but reliant on surface aeration only.
b. Transfer the process to a larger, actively sparged spinner flask, maintain the same high rpm impeller speed to mimic mixing conditions at the smaller volume.
c. Transfer to a stirred tank bioreactor.

Summary

Their simple design, ease of use and low cost make spinner flasks an extremely valuable tool for the development and scale up of cell-based biological processes. The ability of spinner flasks to operate with cell densities that are similar to more complex bioreactors, but that are 1 or 2 orders of magnitude higher than other small scale cell culture systems such as roller bottles, T-flasks, or multi-layer flasks, makes them the ideal intermittent step in cell culture process development.

References.

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Bellco Biotechnology
340 Edrudo Drive
Vineland, NJ, 27006, USA
Tel: +1 856 691 1075
Fax: +1 856 691 3247
Web: www.bellcoglass.com