Perfusion: planning a run

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 an approach for defining operation of a perfusion run using knowledge space, design space, and control space.

Knowledge space

Knowledge space encompasses the known details on process goals and materials sufficient to outline a general scope of work and what is needed to support success. Providing critical process goals such as amount of product needed, product quality requirements, and clinical testing timing, paired with known cell clone behavior and product stability, will help outline practical process scale and operational targets with objective timelines. This can effectively narrow the general process requirements and identify which approaches will work within the knowledge space constraints.

In order to establish an appropriate knowledge space for a perfusion operation, some questions must be answered, such as:

- Does the product have special restrictions (cytotoxicity or stability issues)?
- Are there known quality profile targets (charge variance, glycosylation, fragmentation, or aggregation)?

Ideally, these questions on cell behavior are related to an easily monitored parameter such as target percent cell viability.

To achieve a successful perfusion process, it is important to understand a clone's scaling, which is the functional capacity of a cell line in response to stress such as agitation and gassing at higher operating cell concentrations.

Another critical parameter, target viable cell density (VCD), may be estimated for the selected production reactor volume based on the annual production needs as well as the cell-specific productivity and number of runs that can be completed annually. It will also be important to know approximately what medium exchange rate will be necessary to support your process. This is effectively estimated by knowing your target VCD and cell-specific perfusion rate (CSPR). There are different ways to determine CSPR, such as an iterative flask method or a direct bioreactor method analysis of the early stage of a perfusion run. This direct method analysis of the early stage of a perfusion run, in addition to providing a CSPR estimate, may provide insight on expected cell line behavior such as peak VCD and productivity at high VCD.

Design space

In the design space, necessary equipment and a possible operating range must be defined to support the cell line and process goals as established in the knowledge space, such as product quality and quantity. This is done by determining the best process type to match the production needs identified in the knowledge space.

Establishing the best-fit process is based upon known information about the cell line, product, and facility as gleaned in the knowledge space work. Once the best process type is chosen, the equipment necessary to support that process must be determined. Finally, the practical operating limits for the process can be used to build the design space.

For example, a continuous perfusion process could be optimal for unstable products since those will be removed from the system at a constant rate, resulting in highly viable cell clones. If the clone being used in the process demonstrates highly stable productivity, this scenario would be an obvious best fit for a continuous perfusion process, barring any other considerations or restrictions (Figure 1).

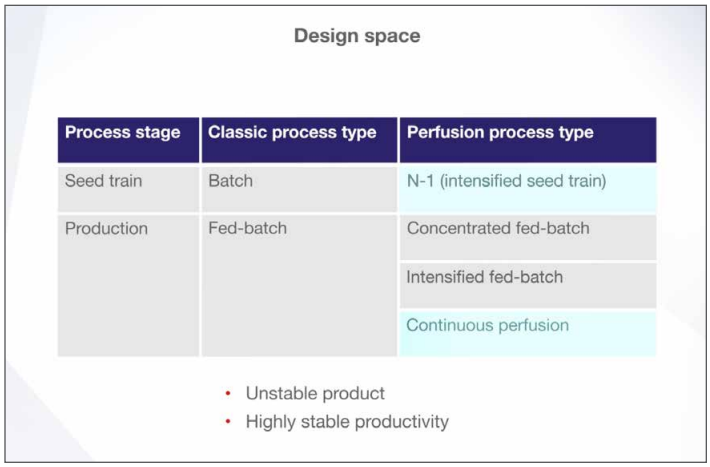

Figure 1. Scenario for a continuous perfusion process.

If there is a need to use existing equipment, such as large production reactors, a classic process type may be the best choice. However, an intensified seed train may still be a good pairing option to improve the overall process (Figure 2).

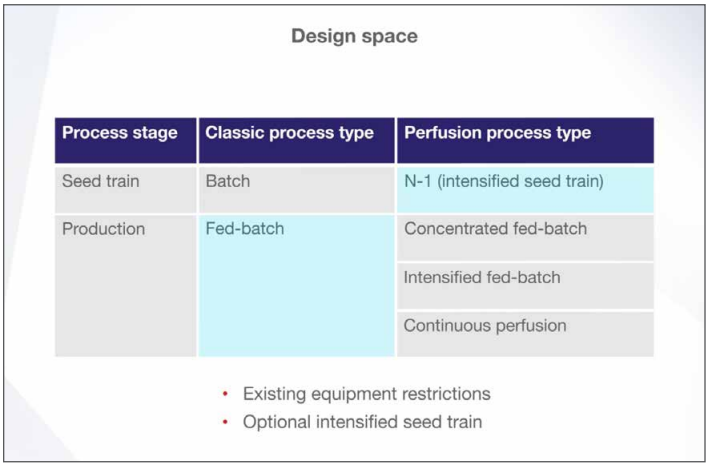

Figure 2. Scenario for a classic fed-batch production process that uses an intensified seed train.

After selecting a process type, the equipment should be chosen to match the process type, the cell line needs, and the annual production output required. Then a production reactor would be selected with an operating volume based on annual production requirements and expected cell line productivity established in the knowledge space. The production reactor would also have to support the process selection requirements. The perfusion process requires compatibility with a cell retention device and a paired controller to meet higher gas flow rates and additional operating hurdles.

Once the process type and equipment are decided, the operating limits of the equipment can be used to outline the design space. This is frequently determined by the capabilities of the production reactor and paired controller, as they are often the most limiting factors. The practical operating range for necessary support of the process and knowledge space requirements must be defined now:

- The final operating volume needs to be adjusted such that the annual titer requirements are met
- The mixing speed of the bioreactor is usually restricted to provide sufficient bulk mixing and mass transfer, or k_La
- The shear rate also needs to be checked based on what the cell line can tolerate
- The oxygen gas flow rate should be low enough to handle initial inoculation, but also support k_La at peak cell density
- And other equipment operating parameters or known critical parameters

Taking these actions with the various operating parameters will help establish the design space.

Control space

For the control space, the operating parameters from the design space must be evaluated by linking their effects to critical quality attributes. Critical operating

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parameters can be identified once initial relationships are established between operating parameters and critical quality attributes. Both process optimization and improved knowledge of the relationship between critical operating parameters and critical process quality attributes can be achieved with successive iterations and analysis between operations. System modeling, digital twins, and other approaches can be used to accelerate progress in optimizing the control space and final process operating targets. This starts by examining the direct operating parameters such as pH and steady-state cell density, scaling parameters such as operating volume and mixing speed, or various other parameters and combinations of parameters.

After evaluating the operating parameters, the important critical quality attributes are considered. These are typically oriented around the amount and quality of product generated, using attributes such as titer, glycosylation, aggregation, or charge species. Next, a design of experiment (DOE) is established to model the effects that the suspected critical operating parameters may have on the critical quality attributes, to determine their relationship.

The above evaluation steps will help you prioritize the operating parameters that are most critical to your process model. The last step is to verify your model and then optimize it through an iterative process. This process should never be considered truly complete, as every execution of the process can continue to feed data that improve the accuracy of a control space design and output consistency that is ultimately achieved.

Summary

Defining the operation of a perfusion run not only sets a foundation for a successful process but also preserves quality and time. Knowledge space, design space, and control space are the three crucial, interdependent platforms that must be explored and reviewed for a successful perfusion process.

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