Significant improvements in bioprocessing in recent decades, including the optimization of cell-culture media to meet desired fed-batch strategies, have advanced industrial monoclonal antibody production to attain levels of productivity never before achieved.

Optimization of cell-culture media, both growth and feed supplements, is a critical and necessary step in maximizing the productivity of biotherapeutic protein manufacturing.

As most biopharmaceutical production systems are based upon fed-batch platforms, it has become common to employ a step-wise approach for cell-culture media optimization by screening for a lead growth medium prior to screening various feed media that can ultimately be paired back with the selected growth medium.

Recent studies from Irvine Scientific suggest there are advantages to simultaneously pairing both growth and feed media over an iterative approach for selecting a fed-batch culture system able to balance viable cell growth, culture longevity, system productivity, and quality of expression product. Data from Irvine Scientific’s internal media development studies is presented here to demonstrate the specific benefits and advantages of a media pairing evaluation approach.

**Evaluating Growth Media**

As the first step in the comparative study between the step-wise and media pairing approach, various growth media were evaluated in batch mode (Figure 1). A CHO cell line producing a biosimilar (maintained in BalanCD© CHO Growth A) was inoculated at a density of $3 \times 10^5$ cells/mL with a 30 mL working volume into 125 mL shake flasks for each growth medium of interest (BalanCD Growth A, Medium 1 and Medium 2).

 Cultures were maintained at 37°C, 5% CO₂ in a humidified incubator and agitated at 120 rpm on an orbital shaker for 14 days. A temperature shift was performed on day 7 to 33°C. Viable cell density and percent viability were determined daily using a Vi-Cell™ XR cell viability analyzer (Beckman Coulter). Glucose levels were monitored every other day using a BioProfile® Flex (Nova Biomedical) and were brought back up to 8 g/L when levels fell below 4 g/L. Antibody titer was quantified on days 10 and 14 using an Octet® QKe from ForteBIO (Pall Life Sciences).

Notably, Medium 1 gave the best performance after 14 days of culture (Figure 1) and would subsequently be selected to advance to the fed-batch evaluation stage, whereas BalanCD CHO Growth A and Medium 2 would not if the step-wise approach was employed.

For the next step in our evaluation, the same set of the growth media (BalanCD Growth A, Medium 1 and...
Medium 2) were evaluated again in fed-batch mode with five different feed media (Figure 2). Similar culture conditions were used from the batch evaluation outlined above. All fed-batch conditions were fed every other day starting on day 3 until day 11 for a total of five feeding events.

Feed A, Feed B, Feed C, and Feed D were fed a total of 30% of the initial culture volume (1.8 mL per event). BalanCD CHO Feed 4 was fed at 20% of the initial culture volume (1.2 mL per event). As observed in Figure 2, the growth media performance ranking noticeably changed after the introduction of feed media, with BalanCD CHO Growth A supplanting Medium 1 as the top performing growth medium candidate.

By evaluating all growth and feed media in pair-wise combinations, the top performing combination was determined to be BalanCD CHO Growth A and Feed 4, as it delivered both optimal cell growth and the highest titer (greater than 6 g/L). This pair yielded a 15% increase in antibody concentration compared to its respective Medium 1 combination.

Notably, if a step-wise approach was used for feed evaluation, the best performing combination would have appeared to be Medium 1 and Feed 4, while the truly optimal combination observed with BalanCD CHO Growth A and Feed 4 would have been missed.

These results emphasize the importance of evaluating the growth and feed media with a media pairing approach as the step-wise approach cannot detect the powerful synergism achieved from the empirically optimal growth and feed media pairing.

**Other Points to Consider**

When evaluating growth and feed media with the media pairing approach, it is recommended to carefully select the appropriate feed volume to minimize the risk of over-feeding, which can lead to inaccurate or misleading results. Once the top 2–3 media pair candidates are identified, additional experiments to optimize the feed volume and schedule for each fed-batch event can lead to the optimal fed-batch culture system.

When working with a large panel of growth media candidates, it is not always possible to perform the evaluation in fed-batch mode. If this is the case, it is recommended to perform a batch evaluation first in order to reduce the candidate list to a more manageable number of growth media to pair with feeds.

During the initial batch evaluation, different selection criteria should be applied, with titer typically being the highest priority throughout the fed-batch evaluation. However, cell growth during the first week of culture might outweigh culture longevity or antibody concentration in importance, depending upon the specific goals of a particular project.

When producing biosimilars or novel biologics with very specific protein quality attributes, the impact of decisions made when devising media combinations can have profound effects on targeted outputs. In addition, it is strongly recommended to choose several growth medium candidates (instead of one) to advance to the fed-batch evaluation in order to increase the likelihood of pairing a compatible feed medium with a given growth medium.

This study highlights the benefits of evaluating media pairs in a fed-batch mode following the general guidelines of media selection methodology. This approach is applicable for media optimization during process development, as well as at the lead clone selection stage in early phases of cell-line development.

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