Achieving consistent performance in bioprocessing through identification of key drivers in media and supplements

Neelanjan Sengupta, PhD, R&D Scientist, Thermo Fisher Scientific; Graziella Piras, PhD, Marketing Manager, Thermo Fisher Scientific

Introduction

The past 30 years have brought significant progress in the design and manufacture of cell culture media as well as in the types of components used. With these advances, the biopharmaceutical industry has improved its processes from producing just a few milligrams of product per liter of culture to ≥10 g/L.

While the industry has achieved higher titers, obtaining consistent protein production and protein quality attributes, such as glycosylation, is critical in today's bioproduction industry, especially for biosimilar molecules. Historically, peptone supplements have been used widely as a basic ingredient of microbial media. In the 1990s, peptones were introduced as a substitute for serum to achieve high expression titers (3–6 g/L). That progress was led by two essential forces: the need and desire to reduce the risk of adventitious agents (e.g., removal of sera due to spongiform encephalopathy contamination in the 1990s) and to achieve better process control for higher product quality, safety, and efficacy.

Further requirements for consistency and safety led to development of chemically defined media and supplements. However, achieving consistent bioproduction targets can be a challenge with both chemically defined and peptone-containing media. Understanding the effects of cell culture media components on the bioproduction process is critical to achieving a more consistent process. This is in alignment with the spirit of the quality by design (QbD) initiative led by the US Food and Drug Administration (FDA).

Major drivers in cell culture

The first important cell culture parameter is the cell line itself: how it is designed, the nature of the expression system, and cell line stability—all of which will influence process variability. The second driver encompasses process parameters for growing cells and controlling bioreactors. The third is the cell culture medium and all components and supplements used. These are essential to producing high-quality proteins that will be safe and efficacious.

While modifications to the cell line and process can require extensive time and effort, optimization and supplementation of cell culture media offers a rapid option for achieving a consistent desired bioproduction outcome. Here we focus on process variability, related to both the media and the components or supplements within them, and our unique solution for leveraging our deep understanding of media to achieve consistency.

Variability in media

With chemically defined media, the assumption might be that because they are made of defined components, they should have less variability. But are chemically defined media truly devoid of variability?

In the past few years, speakers from leading pharmaceutical companies have presented on the impacts that raw-material impurities have had on cell culture performance and product quality in chemically defined media formulations. Trace-metal contaminants coming from salts or amino acids also can impact protein production levels and product quality attributes.

For example, manganese as a trace contaminant in iron sulfate can affect the glycosylation pattern of a monoclonal antibody (mAb). Copper concentrations have also been shown to affect cell culture performance and charge variants of immunoglobulin G (IgG). Hence, chemically defined media made from defined components can have impurities that cause process variability. Impurities in basal media can overshadow natural variations between different lots of peptones, for instance (Figure 1). In every process it is critical to identify the source that can contribute to process variability.

Media using peptones derived from yeast, soy, or other sources to enhance bioproduction can have challenges related to inherent biological variability. Strict quality controls for manufacturing are key to limiting variability in peptones. It is also critical to have process consistency, which depends on base medium components as well as the combination of base medium and supplement/feed composition, and the overall impact of all components should be evaluated.

Achieving consistency: understanding key drivers in media for bioproduction processes

To better control variability and achieve consistency in a specific process, it is important to understand what components in a complete cell culture medium formulation are driving culture performance. A key driver is a component that has a strong positive or negative influence on process performance and must be within an optimal range for cultures to achieve optimal performance (Figure 2).

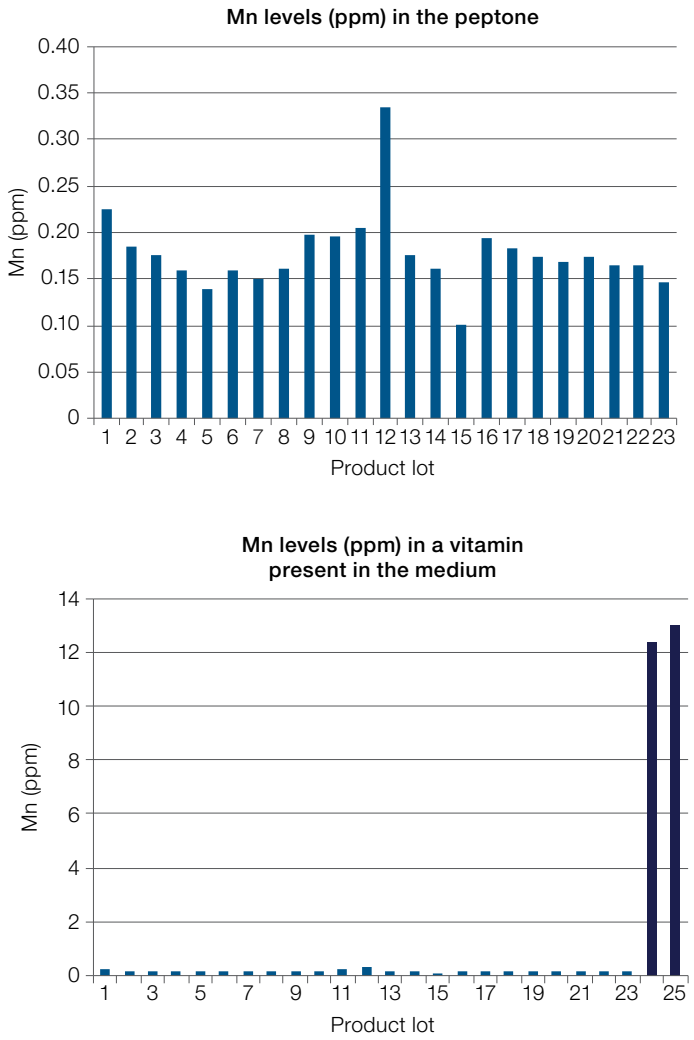

Figure 1. Raw material as a source of variation. In this example, manganese (Mn), which is known to impact protein quality, was found at much higher levels as a trace contaminant in a vitamin present in the medium (12–13 ppm) than in the peptone (0.10–0.35 ppm). In the upper graph, Mn levels are shown for 23 lots of peptone. In the lower graph, Mn levels are shown for the same 23 lots of peptone and 2 lots of media (lots 24 and 25).

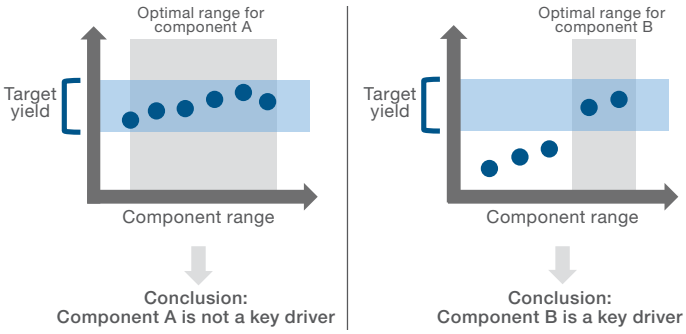

Figure 2. Identifying a key driver component. Key drivers show strong positive or negative influence on performance, have an optimal range to achieve target performance, and cause variability in cell culture performance.

Identifying key drivers

Identifying key drivers that affect process variability in a cell culture process is not as simple as accumulating and examining large amounts of data. To accurately assess which components in a complex medium are driving a process, it is imperative to identify those components showing a statistical correlation of performance across multiple lots of a medium or supplement of interest.

Such analyses are the first step toward identification of key drivers in a bioprocess. Furthermore, parameters that simply correlate with variability need to be differentiated from those that truly cause the variability through statistical analysis and experimental data. Finally, for the best process performance, optimal ranges need to be defined for the key drivers.

In engagements optimizing cell culture media and performance with customers, we have worked to identify the components driving their processes. We have found that keeping nutrients such as nucleosides, vitamins, and trace metals within a very specific range can significantly affect the performance of a cell culture process.

Again, it is important to remember that each process is different, with its own key drivers, even considering when similar base media and supplements are used. Using a statistical methodology will correctly identify key components and their optimal ranges for each specific process.

Predictive modeling

We use a large number of assays to characterize a medium or supplement chemically, identifying and quantifying all of its potential components. The ultimate goal is to build a predictive biostatistical model to predict which lots will perform well in a given process. This biostatistical model uncovers hidden interactions and drivers by including the influence of all components taken together.

With a table of analytical data, we could examine just one component at a time, but the model can consider everything together—reducing a large number of components to just a few key drivers—and ultimately provide a predictive tool for media optimization and screening.

Development of such a model is an iterative process. It requires generating sets of analytical and performance data, then building our unique biostatistical models designed to mimic biological behavior for correlating the analytical and performance data. Further, using proprietary mathematical codes, we can reduce the large number of analytical attributes to top drivers. Several iterations expand the data set to check the model's accuracy and yield a predictive model. These models use different types of mathematical equations—additive, semilog, and multiplicative—to define the relationship between performance and analytical data.

Applying a simple additive model may offer a good model fit but not very good prediction accuracy in some cases. Such scenarios require a more complex modeling strategy, such as a semilog model, to improve the fit and prediction accuracy. A semilog mathematical structure can mimic enzyme kinetics and might better explain a biological process.

The final statistical model carries the risk of overfitting the data, so a particular model might fit better with a small data set but fail as more data are added. As with any statistical model, this modeling approach is highly data dependent; therefore, an iterative approach for model evolution and selection increases robustness and accuracy of the final model.

Using a phased approach

We have established a phased approach to developing the predictive modeling tool used in the key driver identification (KDI) program (Figure 3). To create a statistically sound model, first we work with a customer to accumulate performance and analytical data on at least 5 to 10 lots of cell culture media or supplements.

In phase I, we begin with a small data set (developed in the preliminary phase) to build an initial model. A comprehensive list of analytes is identified for a limited number of lots of media or supplements. These analytes include amino acids, vitamins, carbohydrates, trace elements, and other components present in cell culture media and supplements. This phase yields potential drivers and an initial model that can move to the important second phase.

In phase II, we determine the causative nature of key drivers. Many components can affect performance, but to control process variability, we need to find the key drivers. To achieve that, we create several lots of cell culture media or supplements with enhanced amounts of potential key drivers to determine whether they truly drive (positively or negatively) the yield or quality of a bioprocess.

In phase III, model verification generates a predictive tool to test each cell culture medium or supplement lot. Using new lots of media or supplements, we perform analytical testing to evaluate the model. A customer then evaluates those formulations in a small-scale version of the actual process. If experimental data match the prediction, then we have a locked-down model; if we don't have an exact match, then we may recalibrate the model slightly.

At the conclusion of phase III, we have a predictive tool that allows us to select lots that will provide a desired outcome.

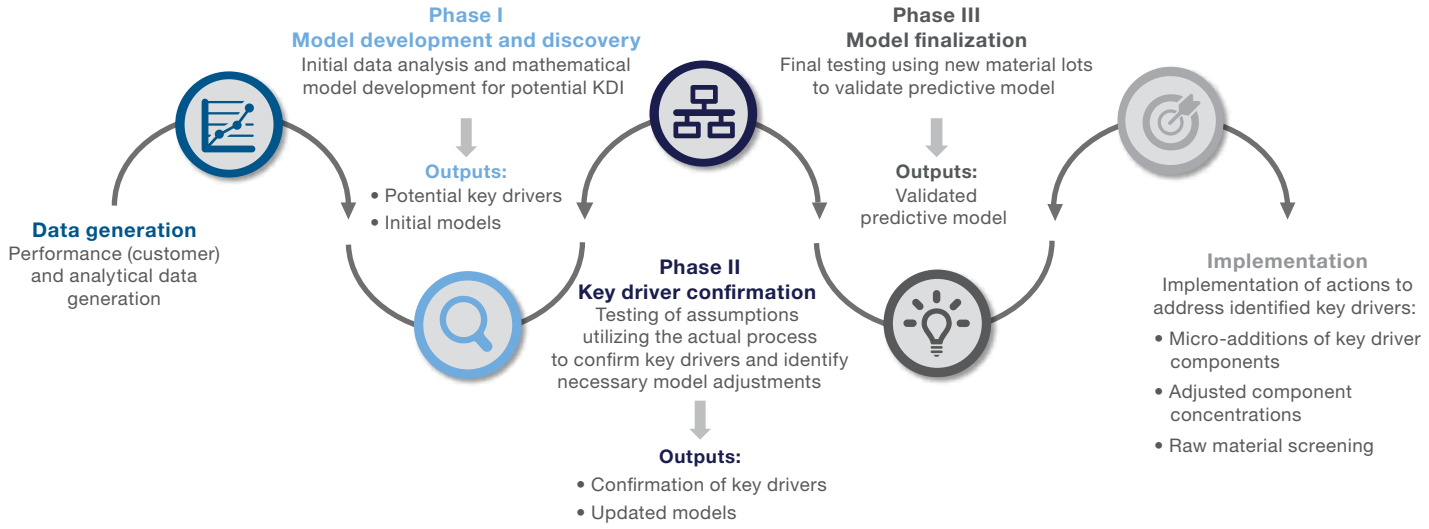

Figure 3. Overview of the KDI program. Proprietary, customizable biostatistical models are the workhorses of the KDI program.

Leveraging media to achieve desired bioproduction goals: chemically defined (CD) media development

Such approaches can be applied to understand the impact of media during the media development process. Here we present an example of such a screen, in which a CHO DHFR cell line was evaluated in 43 CD media, where glycosylation profiles were determined in addition to viable cell density (VCD) and production. Figure 4 shows the total percent galactosylation, which is the sum of all the galactosylated species for each media. The short horizontal bars represent the production for each medium. The VCD is not shown. As seen from the figure, different media result in different production and galactosylation levels.

To further evaluate the impact of media composition, a biostatistical model was fitted in order to screen data for classification and identification of key components in the CD media formulations that have strong correlation to production and galactosylation (Figure 5).

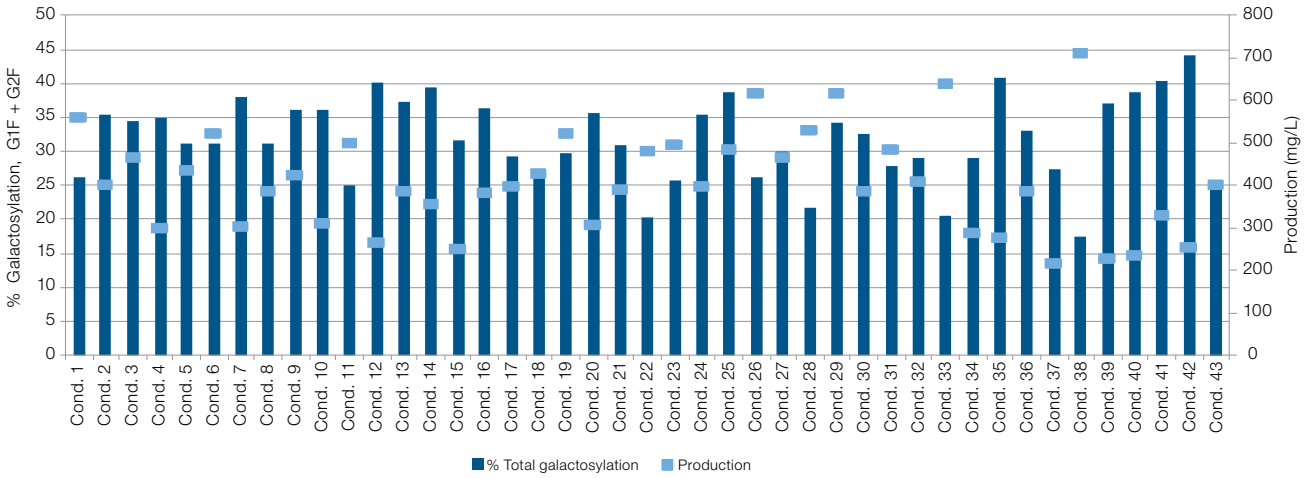

Figure 4. Variation in galactosylation and production as a result of different media. Selection of the optimal medium requires consideration of both mAb production and the desired galactosylation profile.

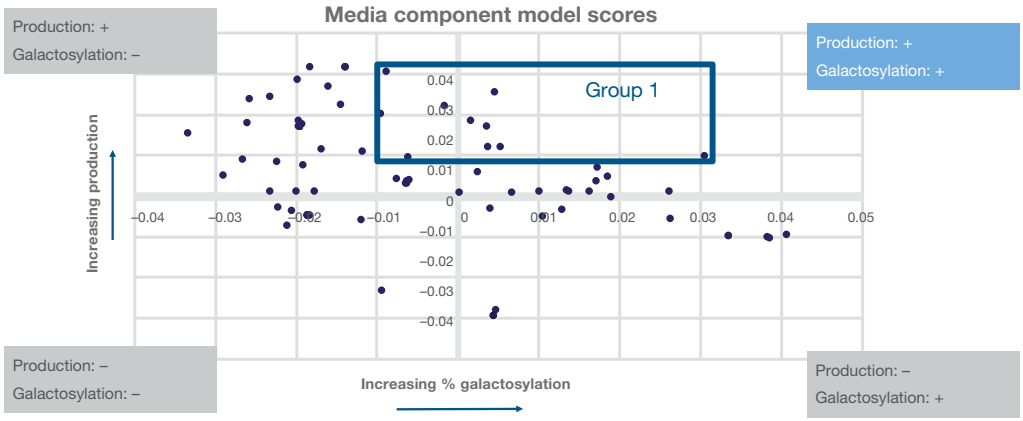

Figure 5. Identifying key components associated with optimal mAb production and galactosylation profiles. Model score plots show the impact of various media components on percent galactosylation and titer. The upper-right quadrant has media components that increase both titer and percent galactosylation. The lower-right quadrant has components that decrease titer, but increase percent galactosylation. The lower-left quadrant has components that decrease both titer and percent galactosylation. The upper-left quadrant has components that increase titer and decrease percent galactosylation.

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A few selected components that had positive correlation with production and minimal negative correlation with galactosylation were identified for further evaluation and were added to condition 42 (a low-production condition) to potentially increase production without having a drastic negative impact on galactosylation. The addition of group 1 components to condition 42 resulted in a 40% increase in production with minimal impact on galactosylation (Figure 6). Hence, such a modeling strategy can provide a targeted approach to media optimization for desired production and protein quality, especially during the media development phase.

Providing consistent performance

Bioprocess variability can be caused by very small changes in specific components or impurities in chemically defined media. Always consider both sources when you're thinking about controlling those variabilities. It's important to build a statistically significant data set to elucidate those components responsible for observed variability.

We have found an effective way to elucidate key drivers from a large data set. It requires an ability to test lots of the media components in a small-scale process so we can correlate that scale with a production process. In addition, we must challenge the model with values of components that are outside the normal ranges to distinguish the causative nature of some components from those that simply correlate. Through predictive mathematical modeling, we can achieve consistent production performance.

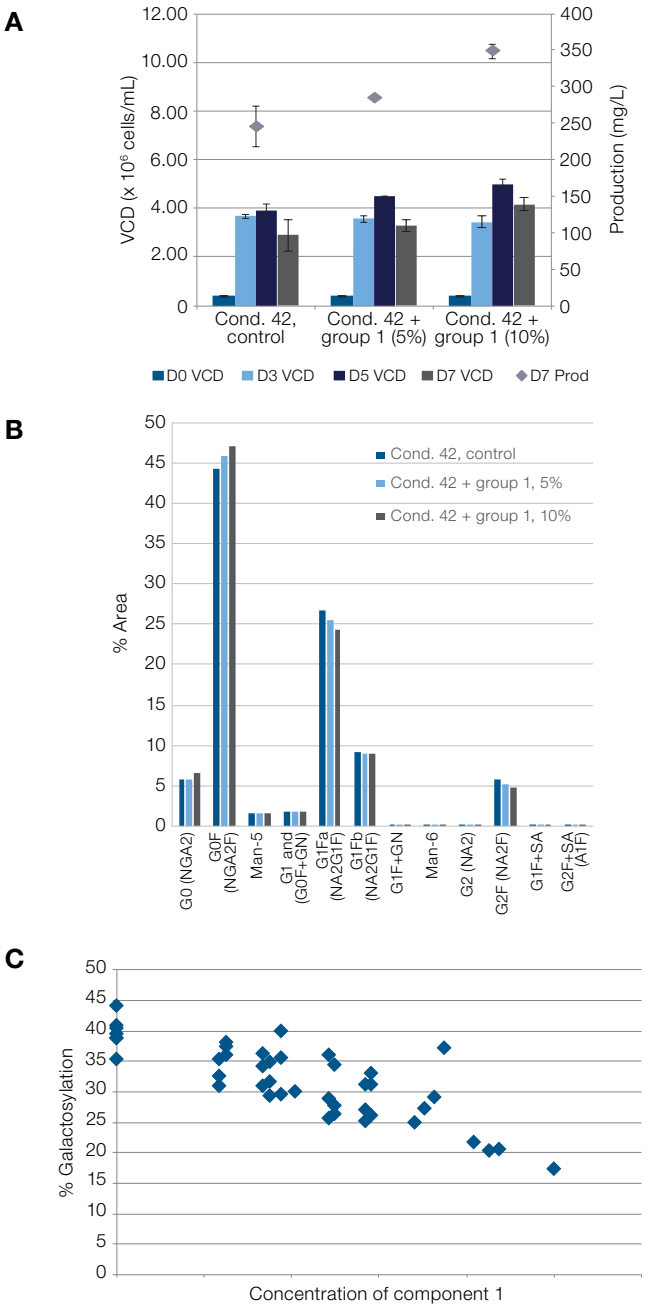

Figure 6. Impact of group 1 components identified in model. **(A)** Addition of group 1 components to condition 42 results in a 40% increase in production. **(B)** Group 1 components have minimal effect on galactosylation. **(C)** A negative correlation is observed between media component 1 and galactosylation.

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