Harness the Power of At-line Spent Media Analysis using the REBEL to Optimize a CHO mAb Fed-Batch Process

Fast and accurate at-line analysis of a bioprocess simplifies and accelerates the selection of the best cell-culture medium and development of feeding strategy.

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OVERVIEW

Cell-culture media (CCM) and feed strategy optimization is a critical step during bioprocessing development. This optimization requires time-consuming processes such as running parallel tests in multiple microbioreactors. However, the low volumes of microbioreactors limit the availability of samples for frequent analysis. Consequently, the bioprocessing industry needs tools that can quickly and accurately analyze the key nutrient consumption to optimize CCM, all with small sample volumes. The following describes a data-driven approach to feed strategy optimization using criteria of productivity, cell growth, and cell viability, whilst reducing the levels of toxic metabolites that might harm product quality.

ANALYZING THE EXTRACELLULAR ENVIRONMENT

By understanding key nutrient trends, a bioprocessing scientist can make decisions on a feeding strategy that is based on measured values—not guesswork or assumptions. Therefore, knowledge of at-line measurements of key nutrients, such as amino acids, is crucial.

For cells to function correctly it is vital that amino acids are available within the CCM, however an excess or lack of these analytes can impact a cell culture's performance. This is referred to as The Goldilocks Problem (see **FIGURE 1**), where the amino acid concentrations within a culture need to correspond to the cell's needs. For example, if the concentration of the amino acid asparagine is too low mis-incorporation of serine can arise in protein sequences. Conversely, the presence of high levels of some amino acids such as glutamine can cause increased levels of toxic metabolites which may negatively affect the charge-variant or even glycosylation profiles of the product [1]. When the amino acids levels are just right, cells are protected from stress, which results in a robust and scalable bioprocess in which cells can thrive, grow, and produce accordingly.

FIGURE 1: Amino acids-A dilemma and opportunity: The Goldilocks Problem.

Traditionally, amino acid analysis requires multiple complex steps, often ones that involve shipping to off-site vendors, and the turnaround time can be 3–6 weeks. With the 908 Devices REBEL at-line analyzer, the analysis can be performed at-line, right beside the bioreactor. The REBEL analyzer has the footprint of a large desktop computer and enables high-throughput measurements with reduced labor and data analysis requirements. For example, it takes only around 10 minutes from sample input to result availability and the sample consumption is minimal—as low as 10 microliters per sample. This allows a bioprocessing scientist to sample and analyze the amino acid concentration of their cultures daily, even when using microbioreactors.

From just 10 microliters of sample, the REBEL analyzes and quantifies all the amino acids and a selection of vitamins within a sample. In addition to being simple to use, the REBEL data can be viewed and analyzed easily. Data can be exported as a .csv file to many different data-analysis tools or in a format that is compatible with the Sartorius Umetrics suite of software tools, such as SIMCA® for multivariate analysis.

ANALYZING CCM PERFORMANCE

Through a collaboration between 908 Devices and CPI, scientists used the REBEL to select the best CCM and optimize the feeding strategy of a GS-CHO monoclonal antibody-expressing cell line. The key goals were improving productivity, enhancing cell viability, and achieving beneficial metabolite profiles within the cell culture.

This ongoing project includes five steps: 1) analyze fresh media for amino-acid content; 2) conduct media screening to find the best CCM for this fed-batch process; 3) use the data from steps 1 and 2 to optimize the cell-feeding strategy; 4) further refine the feeding strategy; and 5) scale up the process to bench scale bioreactors. Currently, steps 1–3 have been completed.

Using the REBEL to analyze a panel of 12 chemically defined CHO CCM (step 1) showed similar initial concentrations of

most, but not all, amino acids within the panel selected. The cells were originally grown in a control CCM and then adapted in shake flasks to the media in step 2 of the project. The results show that the cells adapted successfully in most of the media tested and a range of viable cell densities were observed.

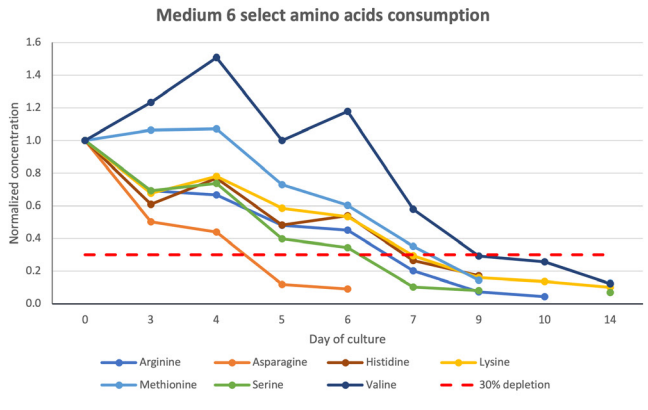
Seven media from the panel plus a control CCM were then used in a screening experiment (step 2) with the GS-CHO mAb-expressing cell line. Forty-eight Sartorius Ambr15® - Advanced Microbioreactor System microbioreactors were seeded for a 14-day fed-batch process. Each microbioreactor was fed with the same low concentration of nutrient feed, and two glucose feeding levels were applied. The microbioreactor conditions were run in triplicate. The analysis consisted of daily measurements of amino acid concentrations using the REBEL. Measurements of cell health (total cell density and cell viability) and cell culture metabolites within the culture (glucose, glutamine, glutamate, lactate, ammonia, and lactate dehydrogenase) were also carried out using the Roche CustomBioTec Cedex Bio HT every other day. The culture osmolality and the offline pH were also measured at set intervals. The mAb titer was measured on day 14 upon culture harvest using the Octet®. During this fed-batch process, medium 6 produced the highest average viable cell concentration and product titer, and medium 9 produced the lowest.

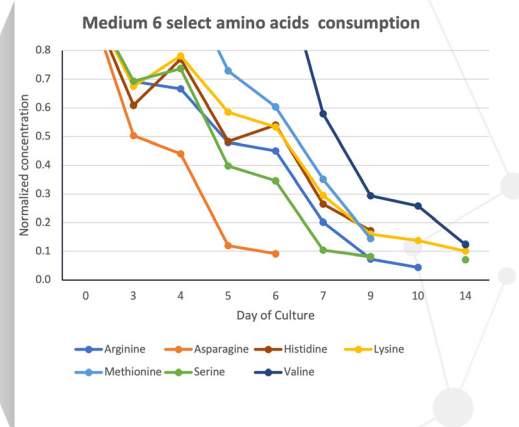
A closer look at data from medium 6 revealed that the glucose-feeding strategies used did not significantly impact cell growth. Cell concentration and viability peaked around day 11, and the latter dropped to around 70% by day 14. In addition, the lactate switched from production to consumption at around day 4 with the concentration being undetectable using the Cedex Bio HT at the harvest time point.

Data from medium 9, the poorest performing CCM, demonstrated some of the lowest cell concentrations and viabilities, dropping to around 30% by day 14. In these cultures, the lactate concentrations did not follow the same trends and the metabolic switch to lactate consumption was not observed. The lactate concentrations remained high and were measured at around 5 g/L upon harvest.

In the bioreactors using medium 6, analysis of the amino acid concentrations within the culture (normalized to levels in unspent medium) showed that many amino acids depleted below 30% of their starting concentration (see **FIGURE 2**). Eliminating this depletion might positively affect cell growth and productivity.

FIGURE 2: Media screening – high performing medium 6.





Despite regular feeding, many amino acids are depleted (below 30% of starting concentration)

No data point means the concentration of the aa has fallen below LOQ (Limit of quantitation) for the REBEL

The same analysis with the low-performing medium 9 revealed that fewer amino acids were significantly depleted. This is likely due to the lower cell growth in these cultures which would reduce the consumption of amino acids. In addition, this result indicated that medium 9 might lack or have too much of a crucial component, beyond amino acids, that limited the cell growth and was out of scope for further investigation within this project.

The results showed the most significant increase in antibody production when amino acids were added to the bioreactors receiving the lowest of nutrient-feed concentrations.

OPTIMIZING A FEEDING STRATEGY

Combining the information acquired in steps 1 and 2, the scientists moved to step 3: refining the amino acid supplementation to meet several key goals. Key goals in this step were to increase the mAb titer while maintaining the cell viability above 60% at the end of the cell culture. In addition, the scientists sought to improve the metabolite profile of the cultures, particularly for toxic metabolites such as ammonia and lactate, and to ensure the cell culture conditions would not have a negative impact on the product quality, such as causing a build-up of undesirable charge variants.

To complete step 3, two high-performing media (media 5 and 6) and two low-performing media (media 4 and 9) were tested in the Ambr15 with a nutrient feed and amino acid supplementation strategy based on data acquired in steps 1 and 2. More specifically, this involved testing 3 concentrations of nutrient feed and 4 concentrations of amino acid mix, which included many of the amino acids that were depleted in previous tests. The results showed the most significant increase in antibody production when

FIGURE 3: The impact of amino-acid supplementation.

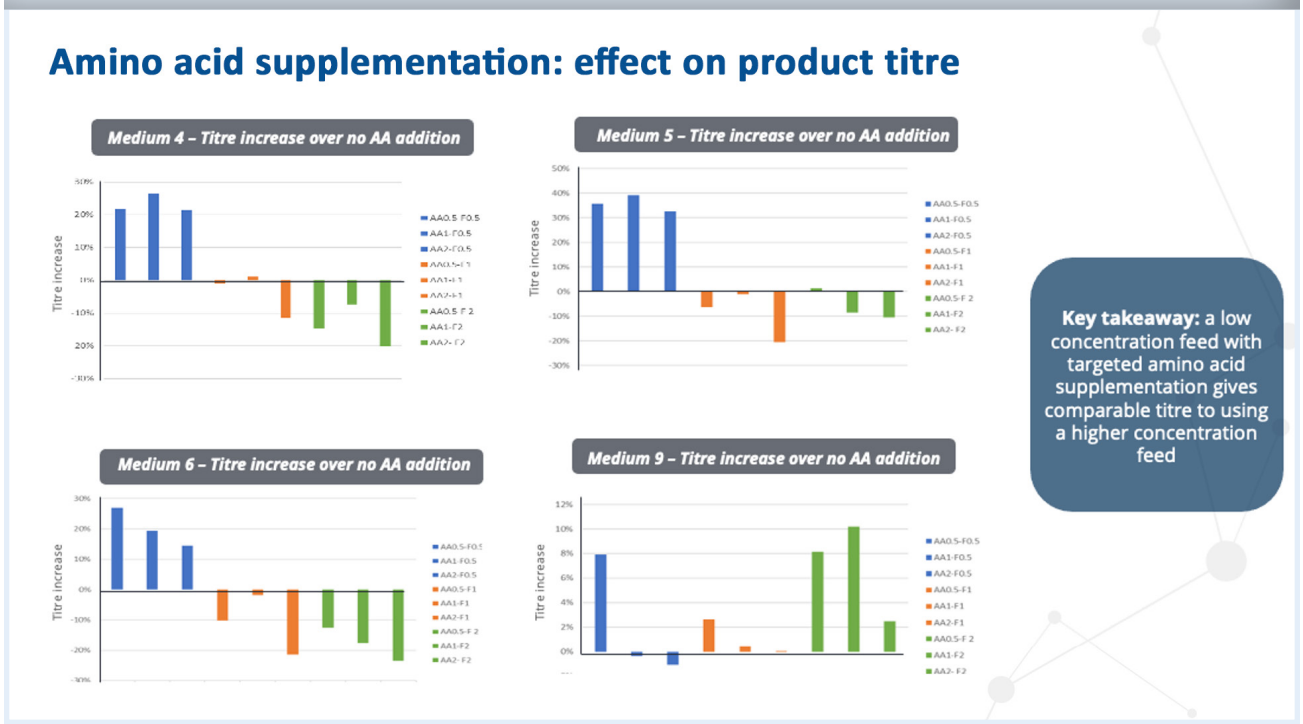
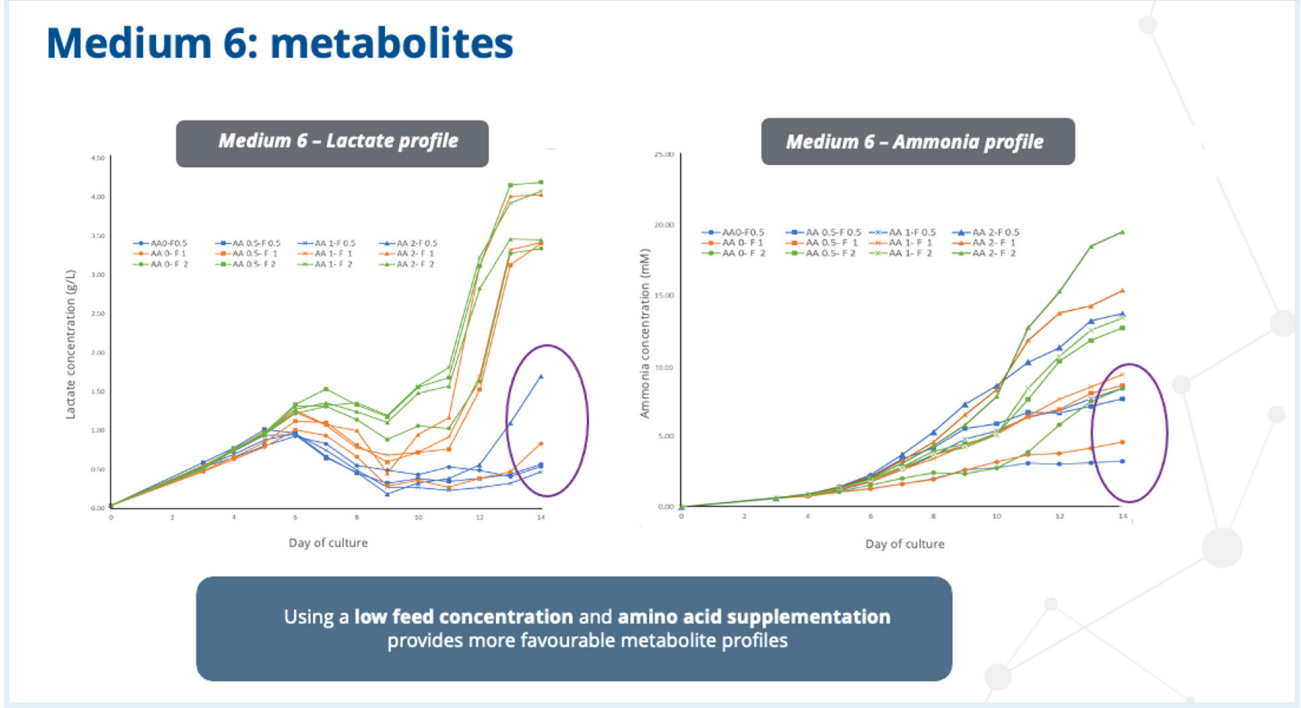


FIGURE 4: Analyzing metabolite profiles.

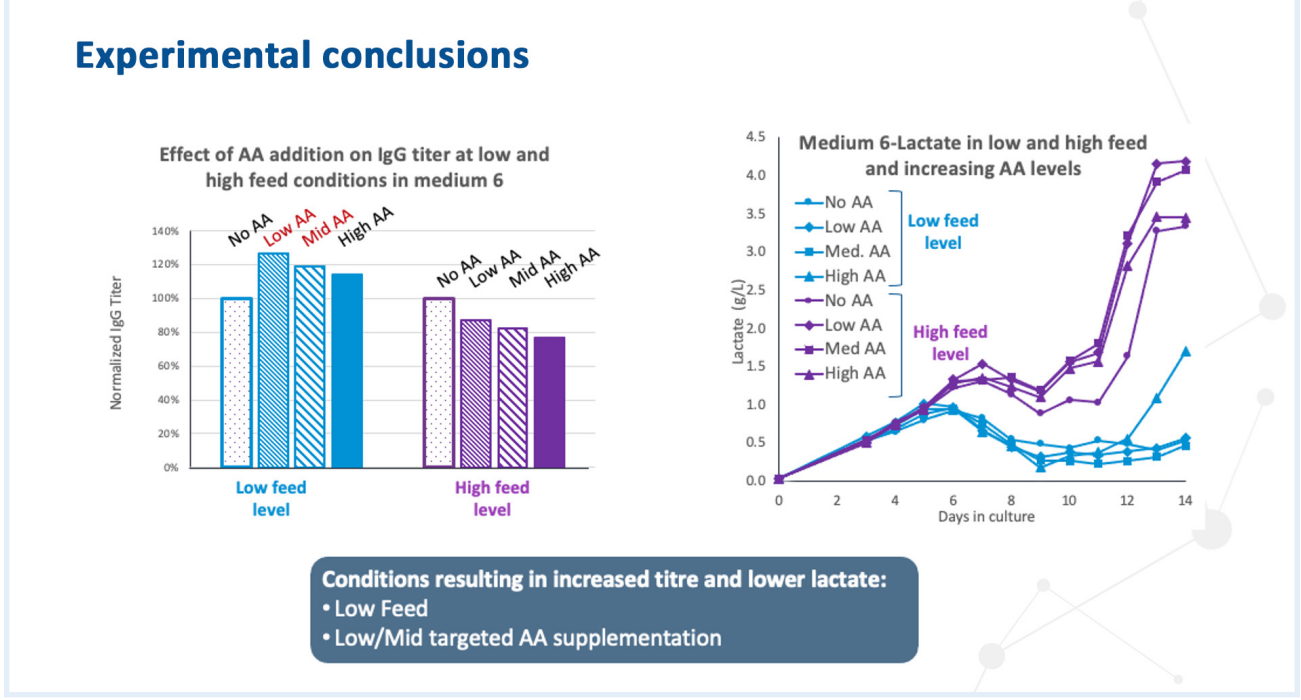


amino acids were added to the bioreactors receiving the lowest of nutrient-feed concentrations. For example, when using medium 5 with the lowest nutrient-feed concentration, amino acid supplementation increased the titer by almost 40% (see **FIGURE 3**). With medium 6, the combination of the lowest nutrient-feed concentration and amino-acid supplementation stabilized the levels of most amino acids over the course of a bioreactor run. Importantly, other key process parameter measurements with medium 6 also showed that a lower nutrient-feed concentration plus amino-acid supplementation improved the metabolite profile, such as lactate and ammonia concentrations in a bioreactor (see **FIGURE 4**). Overall, these results demonstrated the benefits of developing a tailored feeding strategy instead of just adding more of the nutrient feed.

To explore the supplementation landscape, the dataset was further analyzed with MODDE—Sartorius's Design of Experiments (DoE) software—and part of the Umetrics suite of software tools. The results from the MODDE modeling closely matched the experimental findings from steps 1 through 3. Overall, the data showed that bioreactors running on a lower nutrient-feed concentration and targeted low- to mid-level additions of amino acids produced a high antibody titer and importantly, lower levels of lactate (see **FIGURE 5**). In addition, these combinations of feeding and amino-acid supplementation produced the highest cell viability.

Overall, these results demonstrated the benefits of developing a tailored feeding strategy instead of just adding more of the nutrient feed.

FIGURE 5: Lower levels of feed and amino acids produce the highest titers and lowest levels of lactate.



Although steps 4 and 5—refining the feeding strategy and scaling up, respectively—remain to be completed, the results from steps 1–3 demonstrate how a feeding strategy can be optimized using at-line amino acid measurement in addition to traditional metabolite analysis.

CONCLUSION

Using the information from the REBEL and various software packages, a bioprocess scientist can speed up the selection of the best CCM and optimization of the feeding strategy for a specific process. This work requires measuring trends and correcting for depletion or over-accumulation of key nutrients and metabolites. To achieve the desired titer, cell growth, and toxic-metabolite profile, a data-driven strategy can reveal unexpected results, such as a lower nutrient-feed concentration working better than a higher one.

REFERENCE

1. Pereira S, Kildegaard HF, Andersen MR. Impact of CHO Metabolism on Cell Growth and Protein Production: An Overview of Toxic and Inhibiting Metabolites and Nutrients. *Biotechnol J.* 2018 Mar;13(3):e1700499