Data-Driven CHO Bioprocess Optimization and Scale-Up with At-Line Intelligence of Key Media Components

Application Note

Repligen Corporation now owns the life sciences PAT product portfolio of 908 Devices Inc. Please contact Repligen for further inquiries.

Highlights

Accelerate process development with fast, at-line spent -media analysis:

- REBEL analyzes amino acids in minutes and fits on the lab bench. Locally performed fast analysis reduces time between bioprocess development experiments.

Achieve target titer while controlling toxic metabolites:

- REBEL at-line analyzer for amino acids, in combination with standard metabolite analysis, can be used to optimize nutrient feeding and control toxic metabolite production, thus improving cell viability and process performance. Titer was increased 30% in optimized conditions, and in the final scale, a final adjustment of one amino acid increased the titer by 6%.

Control cost of goods:

- By using less complex chemically defined feed and targeting specific nutrient needs instead.
- Utilizing fast at-line spent media analysis instead of outsourcing key component analysis.

Scale-up from Ambr15 microbioreactors to 10L bioreactors with optimized nutrient and metabolite profiles, while obtaining target yield.

Introduction

CHO is the most widely used and well-understood cell type for monoclonal antibody (mAb) production. However, each cell line and clone has its own nutritional requirements. In many fed-batch approaches, the cell culture media, basal, and feed, are designed to supply enough nutrients for the majority of cell lines, but overfeeding may lead to the accumulation of toxic metabolites, such as lactate and ammonium,[1] plus contribute to the high the cost of production and thus high cost of goods sold (COGS).

Our initial hypothesis was that using a lower-than-recommended concentration of a commercially available feed would reduce the cost of goods, and make the process more robust by improving the toxic metabolite profile, while maintaining or increasing titer. We had several goals for this project:

- Avoid high lactate and ammonium profiles, which have been shown to alter product quality attributes[2] such as charge variants and glycosylation profiles.
- Avoid amino acid depletion by supplementing amino acids as required: amino acid depletion may cause misincorporations, which may be difficult to detect and correlate to potential product quality and safety impacts.
- Maintain 60%+ cell viability to reduce the risk of damage to the product in the later days of the process,[3] and simulate a more optimal starting point for the downstream process.
- Reach target titer while reducing the cost of goods by optimizing feed strategy.

REPLIGEN

The process optimization and scale-up experiments described here were powered by REBEL Amino Acid Analyzer (see Figure 1). REBEL device performance and usability were recently assessed by FDA contributors.[4] In this project, the cell culture performance criteria selected were viability, production, and metabolite profiles.

The steps were as follows:

1. Fresh media analysis to uncover amino acid content of a chemically defined (CD) CHO basal media panel
2. Media screening to select the best-performing media from a panel for CHO fed-batch process and to determine depletion/accumulation of nutrients and metabolites. This work was done in the Sartorius Ambr15 microbioreactor system.
3. Media/Feed optimization: we investigated the ideal CD feed levels and added a separate mix of amino acids that were depleting during the cell culture. The work was conducted in the Ambr15 system.
4. Feed strategy refinement: to optimize CD feed levels and timing for best performance, refining amino acid mix components and concentrations. The work was conducted in the Ambr15 system.
5. Scaling up: keeping the titer, cell viability and nutrient/metabolite profiles on target, first into the Sartorius Ambr250 system, and thereafter to the final scale of 10L stirred tank bioreactors. In this phase we also made final adjustments to the amino acid feeding.

Materials and Methods

1. Materials

- **Producer cell line:** Monoclonal antibody-expressing GS-CHO cell line (in-house, CPI, Darlington, UK).
- **Cell culture media:** Gibco CHO Media Panel from Thermo Fisher Scientific, CD Feed Efficient Feed C+ (Thermo Fisher Scientific). The CD feed recommended concentration is “2x” with 4% v/v additions every other day. Our experimental design included lower levels (0.5x; 1x) of this CD feed.
- **Glucose feeding:** Bolus feeding: 2 to 4 g/L (or, 4 to 6 g/L in the first Ambr15 run only). In the last step of the project in the 10L stir tank bioreactor, an additional, continuous/dynamic glucose feeding approach was tested, enabled by MAVEN (Repligen).
- **Amino acid mix:** pure amino acids (in powder form; Sigma-Aldrich) were dissolved in water or PBS.

REBEL-Assisted Feeding Strategy Optimization (Experimental Design)

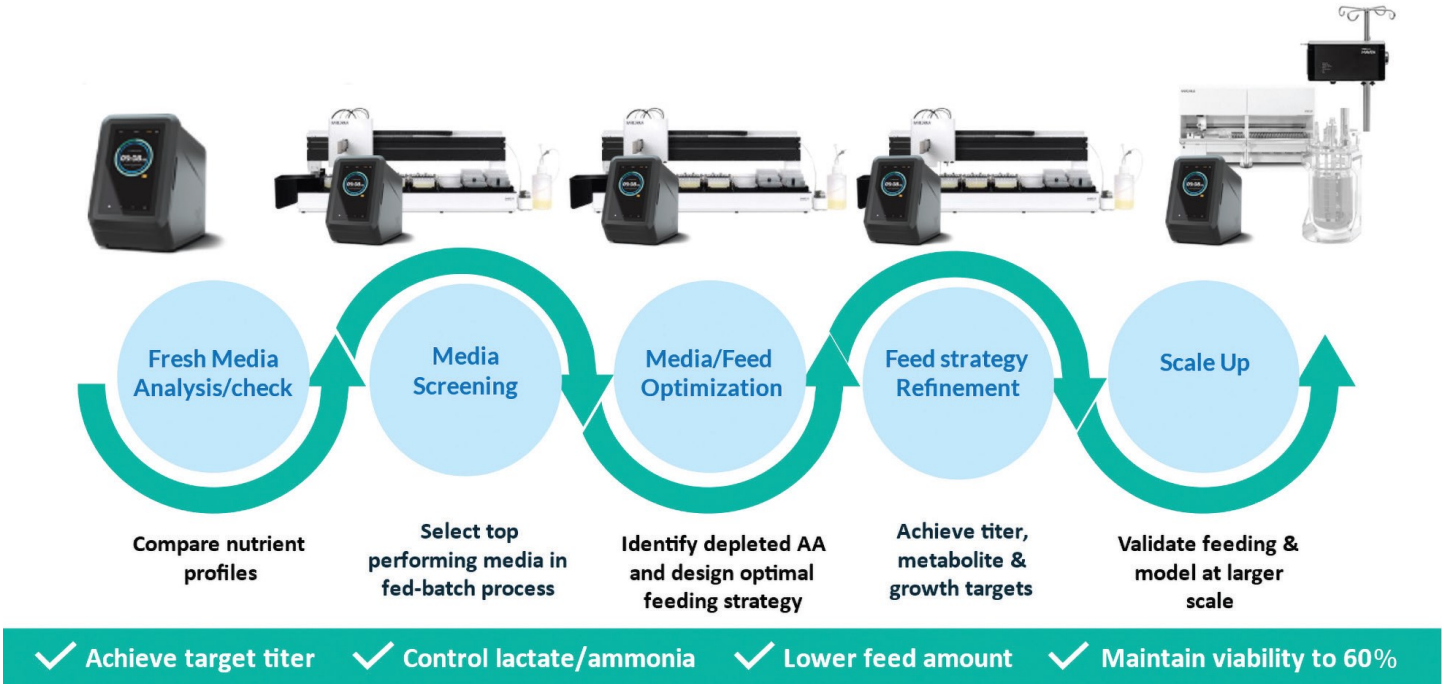

Figure 1. Bioprocess optimization project experimental design. REBEL measurements helped drive our feed strategy optimization and ultimately reach project goals.

2. Bioprocess Equipment and Conditions

- Bioreactors:
  - Sartorius Ambr15 with 48 microbioreactors
  - Sartorius Ambr250 with 24 microbioreactors
  - 10L bioreactors: Biostat 10L stirred tank bioreactors (Sartorius Biostat B-DCU II with BioPAT DCU Tower).
- Conditions: At all scales, the process was a 14-day fed-batch with daily glucose feeding if needed and commercial feed every other day (different strategies applied; please see results and discussion). Cells were seeded at 0.3 million cells/mL and grown at 37°C. The dissolved oxygen set point was 30% and the pH set point was 6.9, with a deadband of 0.03.

3. Analytical equipment

- At-line analytics:
  - Standard metabolites: Cedex Bio HT Analyzer (Roche) for glucose, lactate, ammonium, LDH, Gln, Glu.
  - Cell density and viability: Vi-CELL (Beckman Coulter).
  - Osmolality: 3320 Osmometer (Advanced Instruments).
  - Amino acids: REBEL (Repligen).
  - 10L bioreactor: on-line glucose and lactate monitoring with MAVEN (Repligen) biosensor-based real-time measurements
- In-line and on-line analytics: pH, Temp, CO_2, O_2 as provided in the Sartorius control system in the bioreactors used (see above)
- Off-line product quality analysis:
  - Titer: Octet Red384 (Sartorius)
  - Glycans: Labchip GXII Touch system (Perkin Elmer) with the glycan release and labeling kit and reagent kit (Perkin Elmer)
  - Charge variant analysis (cIEF): iCE3 (Protein Simple)

4. Sample handling for bioprocess analytics

- Samples were automatically pulled from the Ambr15 and Ambr250 using the Ambr device. Samples from the 10L bioreactors were pulled manually.
- For Vi-CELL, samples were taken directly to analysis, and for Cedex, the samples were prepared by sample dilution and centrifugation when needed.
- For REBEL, the samples were filtered and diluted with the provided diluent. The first Ambr15 experiments were analyzed with dilution factor 200, and the following (including Ambr250 and 10L bioreactors) with dilution factor of 150. These tasks were performed on a 96-well plate.

5. Software for data processing

JMP statistical software with JMP add-in was used to visualize the rich data sets from REBEL spent media analysis. Bioprocess at-line and off-line data were processed with vendor-provided specific software packages, and collated in Microsoft Excel.

Sartorius Umetrics suite of software products SIMCA and MODDE were used at the Ambr15 level to support experimental design.

Procedures and Results

1. Fresh media analysis

A panel of basal chemically defined (CD) media (Thermo Fisher Scientific) for CHO cell culture were analyzed with REBEL for nutrient content (amino acids and select vitamins). Media were tested to select a diverse set of media compositions for media screening. Previous AA consumption profiles in these media were not available for this cell line.

Figure 2 shows amino acid concentrations measured with REBEL for the media tested. None of the media tested contained glutamine or significant amounts of alanine or glycine. Medium 1 shows highest levels of amino acids such as aspartic acid, leucine, proline, serine and valine. In order to screen a diverse set of media, media 2, 4, 5, 6, 7, 8, 9 were selected for the screening. **When performing a media screening experiment using a panel of media, REBEL provides information about media nutrient diversity, enabling informed decisions about which media to test.**

2. Media Screening

Media screening was performed in the Sartorius Ambr15 using a full 14-day batch-fed process. We screened eight media at two glucose feeding concentrations with daily glucose measurements and feeding as required:

- *Low glucose* feed (2-4 g/L): when glucose fell below 2 g/L it was supplemented back to 4 g/L.
- *High glucose* feed (4-6 g/L): when falling below 4 g/L, glucose was supplemented to 6 g/L.

A total of 16 conditions were run in triplicate bioreactors for total of 48 bioreactors. The goal was to establish the correlation between the usual performance (titer and growth) levels and the nutrient (amino acid) consumption.

Feeding with low concentration (0.5x instead of manufacturer -recommended 2x) of chemically defined (CD) feed every other day began on day 3. Low CD feed concentrations were used to keep metabolite (lactate and ammonium) profiles under control and thereafter reveal potential AA depletion in

Amino Acid Levels in Media Panel

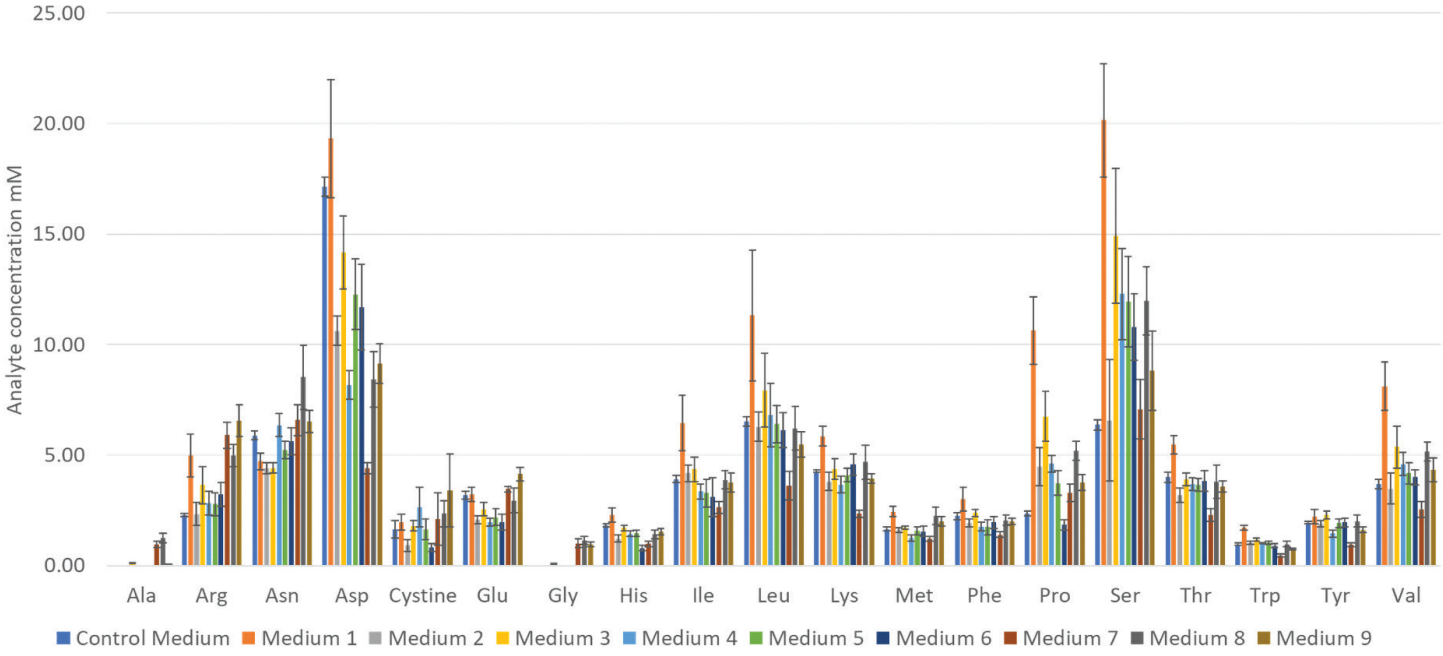

Figure 2. Chemically defined CHO cell culture media panel amino acid concentrations as measured by REBEL.

these conditions. Standard metabolites, cell density/viability, glutamine/glutamate, and osmolality measurements were performed every other day. REBEL amino acid analysis was performed daily, and titer was measured on selected days.

Medium 6 was the best performing medium based on cell growth and titer (see Figures 3A and 3B), and was used in all following stages as the best candidate. In addition, three other media (4, 5, and 9) were taken to the second stage (Ambr 15). The results showed no significant differences between the two glucose feeding strategies, thus the team chose the lower glucose feeding regime for all further experiments. Amino acid consumption (in mM), based on REBEL daily measurements, were calculated for each cell culture medium taken forward.

With this low feed regime, several amino acids were depleted as shown in Figure 3C, some before the midpoint of the cell culture time course. In medium 9 (low-performing medium with this cell line) the amino acids were not consumed at the same rate as in the other, better-performing media. Slower consumption of amino acids correlates with lower VCD with this medium. **With daily REBEL measurements, we were able to capture all amino acid trends throughout the bioprocess for all 48 microbioreactors without losing bioreactor volume in the Ambr15. The analysis was performed at-line, next to the bioreactor.**

REBEL results are reported as concentrations (mM) which allows the measured values to be directly used for calculating consumption rates. These values can then be directly used to identify which amino acids, and in which concentrations to add, to avoid depletion of these key nutrients. **JMP statistical software, with JMP add-in, was used to display REBEL data, enabling quick visualization and analysis of amino acid trends, in parallel with microbioreactor experiments** (Figure 3C).

3. Feeding strategy optimization

Feeding strategy optimization was performed in the Sartorius Ambr15 -48 vessels using a full 14-day batch-fed process. A matrix of conditions (DoE in Table 1) was used to optimize the

Table 1. Media feed strategy optimization experiment DoE with four different media (4, 5, 6, and 9) with CD feed levels (0.5x, 1x and 2x) and amino acid mix levels (0, 0.5x, 1x, 2x).

	CD Feed/AA mix		
For each media (Medium 4, 5, 6, and 9) the same CD feed and AA mix combinations were used	0.5x / 0x	1x / 0x	2x / 0x
	0.5x / 0.5	1x / 0.5x	2x / 0.5x
	0.5x / 1x	1x / 1x	2x / 1x
	0.5x /2x	1x / 2x	2x / 2x

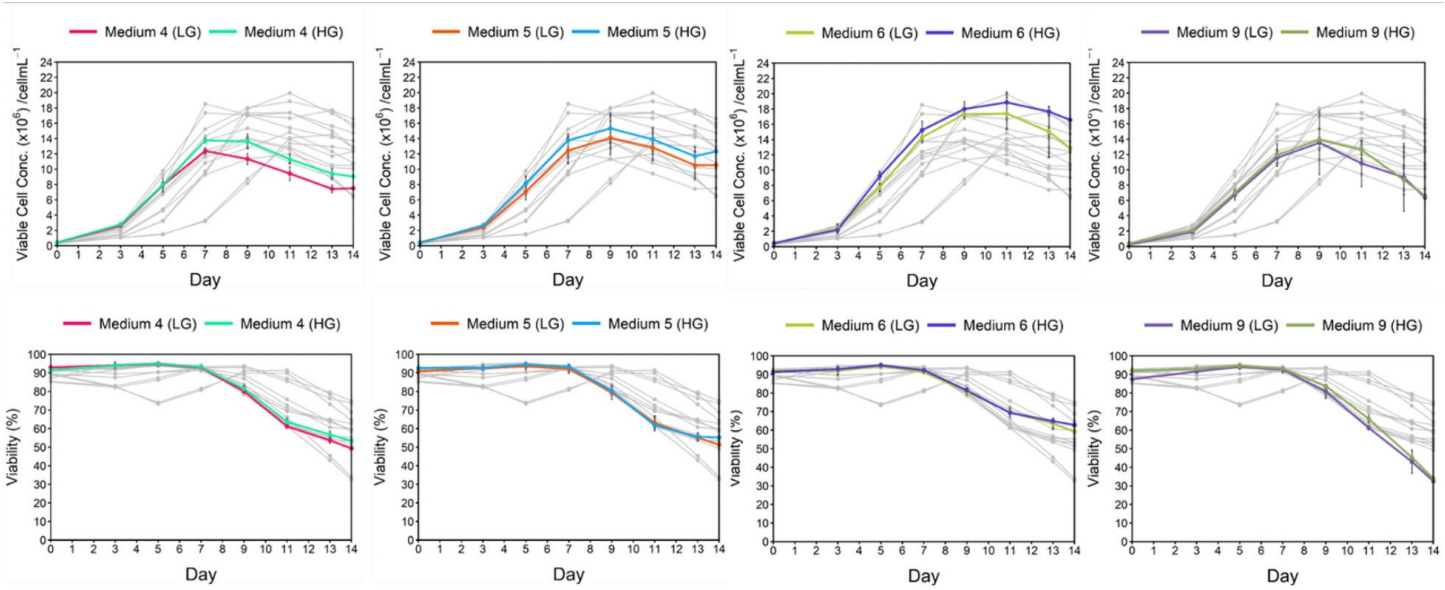

Figure 3A. Media screening experiment in Ambr 15. Cell growth and viability for select 14-day cultures using cell culture media 4, 5, 6 and 9 from the panel. High glucose (HG) and low glucose (LG) feeding conditions were tested with all media. Each condition was done in triplicates.

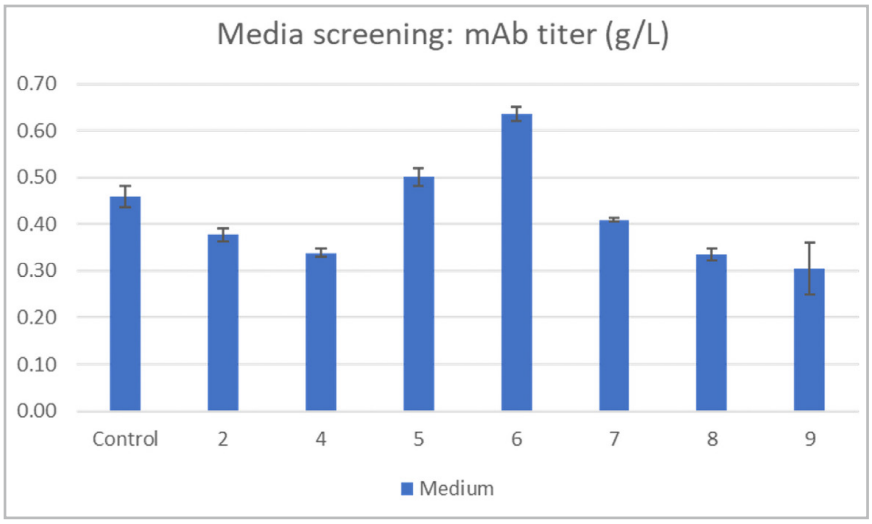

Figure 3B. Day 14 (average) titer for all cell culture media tested in the media screening experiment. Each medium in the media panel was run in low glucose and high glucose conditions, and all conditions in triplicate.

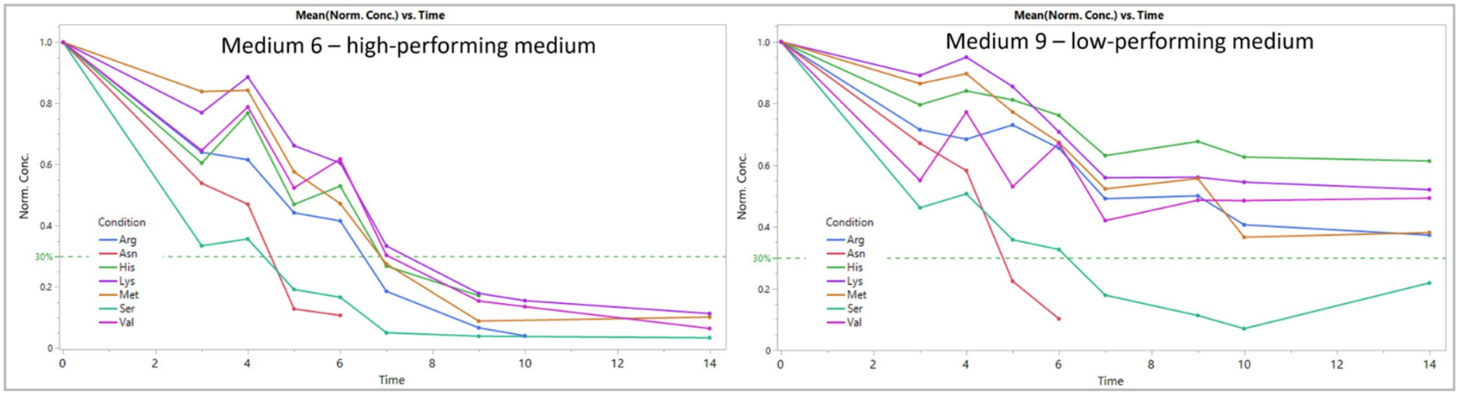

Figure 3C. Select amino acid trends in panel media 6 and 9 from the media screening stage. All amino concentrations are normalized to day 0—basal media concentration—to better visualize amino acid consumption trends.

feeding strategy in media 4, 5, 6 and 9 with the goal of achieving target titer while keeping toxic metabolites (lactate and ammonium) low. This matrix included the manufacturer-recommended level of the CD feed ("2x") and two lower feed levels "1x" and "0.5x". A group of amino acids, the AA mix, containing arginine, asparagine, histidine, lysine, methionine, serine, valine identified in the media screening step as being depleted (see Figure 3C) were added to the feed at three different levels as well. The concentrations of added amino acids were calculated from the consumption rates obtained by daily REBEL measurements in the media screening step.

Figure 4 shows the approach taken for remedying the depletion of amino acids. The concentration of each amino acid in the AA mix was calculated based on the consumption, to feed the amino acid back to the concentration found in the fresh basal media (bi-daily feeding), and was called level "1x". We added the levels of half ("0.5x") and double ("2x") the concentration for each amino acid in the mix, respectively, to have a wider experiment range. Controls with no amino acids, only CD feed added, were also run.

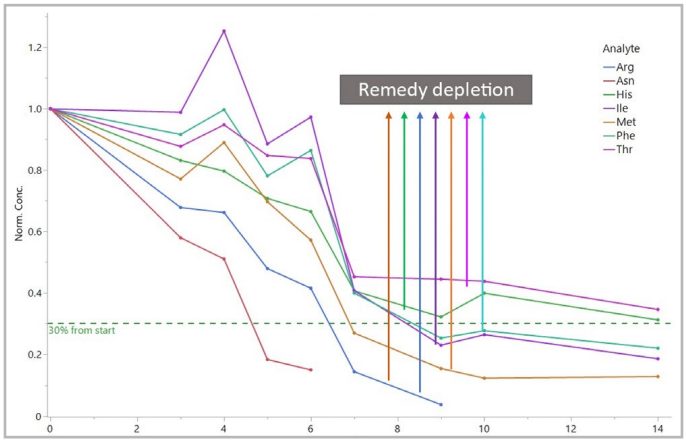

Figure 4. Normalized concentration of the 7 amino acids measured with REBEL and showing depletion in medium 6 in the media screening step. This data was used to determine the concentrations of amino acids to add as an amino acid mix in the feed strategy optimization step. The aim was to keep the amino acid concentration close to the level found in the fresh basal media and avoid depletion. REBEL provides actual concentrations (in mM), but here, for demonstration purposes, the data has been normalized to the starting concentration (basal medium level).

Figure 5 shows the impact of adding the AA Mix to the low feed condition (0.5x) on titer (5A), lactate (5B), ammonium (5C) and cell viability (5D) in the feed strategy optimization step. Medium 5 showed the highest impact on titer improvement upon amino acid bolus addition to the feed as compared to the CD feed alone.

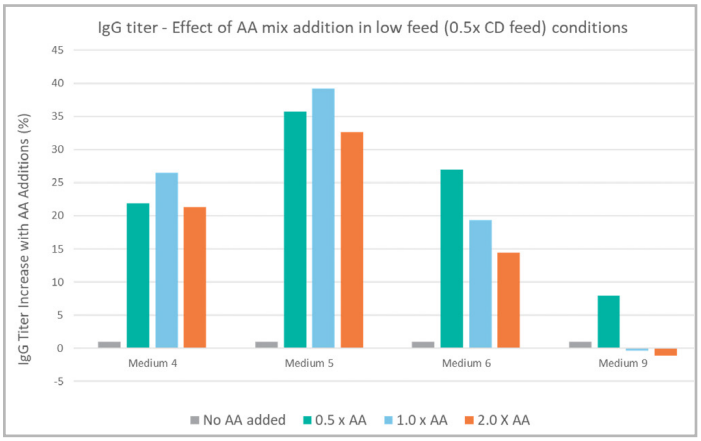

Figure 5A. Feeding strategy optimization: Titer improvement in four different media, only in low level (0.5x) CD feed conditions, with four levels of amino acid mix. The data here is normalized for each media to the No AA mix condition (=100%). The titer increase is the highest in medium 5 (over 30%), and no benefit of adding AA was seen in medium 9. Overall, the highest titers (in g/L) were produced in medium 6 (data not shown).

We were able to make several noteworthy observations on the feeding strategies and media used based on analysis made possible by REBEL:

- The level of supplementation of amino acids that seemed to work best was the one that matched the starting concentration of the compound in basal media ("1x"; Medium 4 and Medium 5) or slightly less ("0.5x"; Medium 6).
- In all cases, adding too much ("2x") of amino acids was the least beneficial in any of the tested conditions.
- One basal medium (Medium 9) did not show significant improvement in titer from the amino acid addition, suggesting this medium is lacking other key nutrients.
- Adding extra amino acids at higher CD feed levels did not improve the titer (data not shown); however, the overall metabolite profiles showed less favorable trends when using high CD feed levels as shown in Figure 5B. Lactate and ammonium levels were elevated in the manufacturer -recommended CD feeding regime, with 2x level feeding every other day.
- Titer in condition 0.5x CD feed with AA mix added, was similar to the titer obtained with the high feeding strategy recommended by the manufacturer. However, lactate and ammonium profiles were significantly lower in the low feed with select amino acids added (Figures 5B and 5C) than in the control.

REBEL-enabled quick optimization of the process by determining the right level of nutrients to achieve target titer while lowering toxic metabolites.

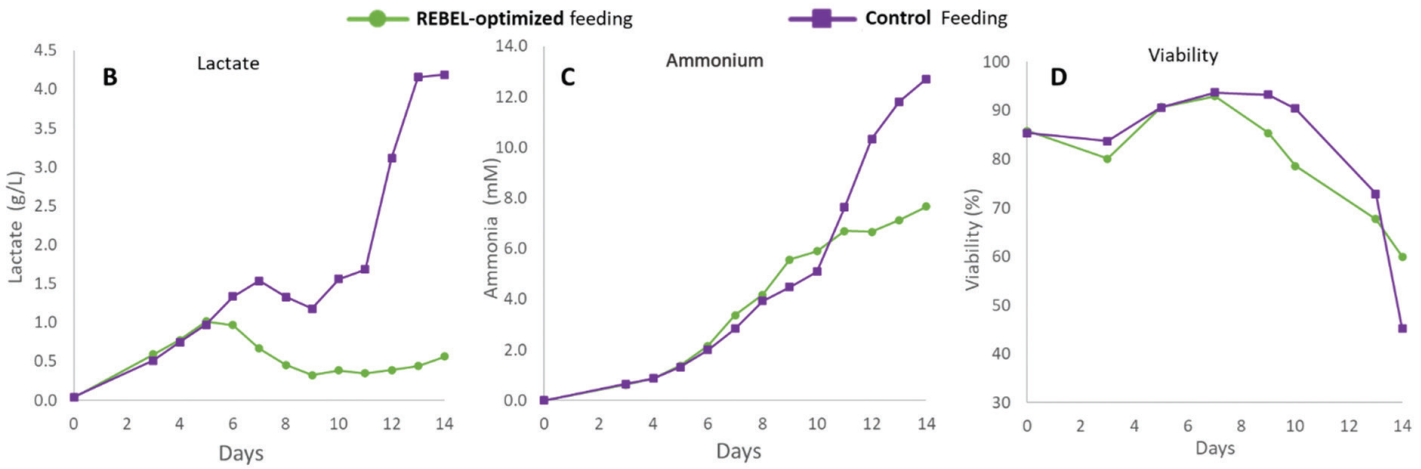

Figure 5. B) lactate C) ammonium and D) cell viability in select conditions in feed optimization experiment. Lactate and ammonium concentrations in the control (high level of CD feed) show significant higher levels than in the REBEL-optimized feeding condition. The REBELoptimized feeding strategy uses a lower level CD feed with extra amino acids added based on observed amino acid consumption. Viability decreases in last days in both conditions, to 60% for the REBEL-optimized feeding, and below 50% (45%) for the control feeding condition.

4. Feed Strategy Refinement

The feed strategy was further refined by fine-tuning the feeding volume and schedule: we compared a flat bi-daily feeding with a constant volume (0.5x CD Feed), or pyramid feeding conditions (Table 2), starting day 2 or 3. In an Ambr15 run with 48 vessels, it was shown that the pyramid feeding was contributed positively with titer (Figure 6). We experimented with additions of amino acids in different combinations, however, from the conditions tested, Sartorius MODDE (part of Umetrics suite of software) supported the positive correlation of pyramid feeding strategy being the most significant contributor to titer. In Figure 6, all four CD feeding strategies are compared with control (G1—no amino acid mix added) and a mix (G1-3) of three amino acids added in addition to the CD feed.

In the scale-up (Ambr250 - 20 vessels), we continued with pyramid feeding strategy since that was effective in earlier rounds, but tested two different amino acid mixes:

- A mix of seven essential only amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, valine), that had been used successfully in other experiments.
- A refined amino acid mix that included both essential and non-essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, and valine).

Whereas some branch-chained amino acids (e.g., leucine, isoleucine) may need to be controlled to a low level to avoid growth-inhibiting effects,[2] other non-essential amino acids may not be required in feed to match consumption. An example of this is asparagine, which may, in GS CHO cell lines, be converted directly into ammonium[2] with negative effects to the metabolite profile we wanted to control. An example of this is shown in Figure 5C, green trace, where the ammonium profile for the REBEL-guided feeding strategy was

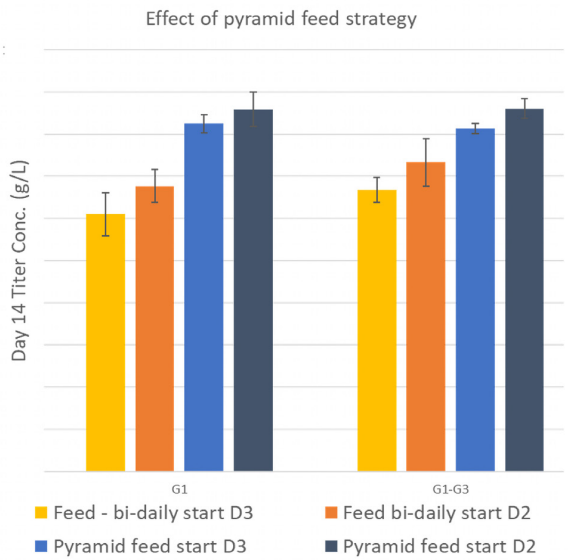

Figure 6. Effect of pyramid feeding in two groups of bioreactors in the feed strategy refinement experiment in Ambr15 system. The yellow and orange bars indicate day-14 titers for bi-daily (every other day) 4% (v/v) additions of CD feed, and the blue bars indicate titers for every other day pyramid feeding (variable volumes, see Table 2) CD feed additions. Addition of certain amino acids (G1-G3) did not significantly improve the titer over control (G1).

still higher than what we had aimed for, albeit being significantly lower than for the vendor-recommended CD feed level cell cultures.

The fed-batch processes in this scale showed similar amino acid depletion profiles as before. Essential only amino acids added as a supplement mix gave the same response as a mix with both non-essential and essential amino acids (adding arginine, serine; Figure 7). We therefore concluded that in this cell culture media and CD feed, the non-essential amino acids depleting below 30% during the cell culture were not required in the additional amino acid mix.

REBEL provided timely analysis of spent media, allowing us to see which amino acids were being depleted and how quickly. The speed of analysis provided by REBEL also allowed us to see how all amino acids behaved across all conditions.

In the Ambr250 experiment, we noticed in the daily REBEL data that tryptophan, an essential amino acid, was depleting. This amino acid had not been considered for addition before, although it had been among the depleting ones. In Figure 8, tryptophan time course trends are shown for select (pyramid CD bolus feed strategy) bioreactors. Only the bioreactors which received 2x level of CD feed maintained tryptophan at approximately starting level, and in all lower CD feed level (0.5x, 1x) this amino acid depleted below 30%, in some cases by day 8. This observation would inform the subsequent scale -up experiments (see next section).

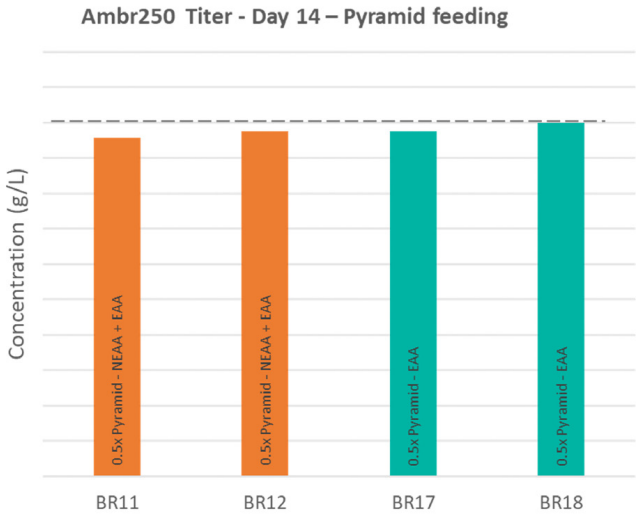

Figure 7. Scale up to Ambr250 and feed strategy refinement experiment: Comparison of adding non-essential and essential amino acids (in orange) to adding essential amino acids only (in teal) together with low level CD feed.

5. Bioprocess Scale-up to 10L Stirred-tank Bioreactors & Introduction of MAVEN

Our process optimization was initially performed on Amb15 runs, in which we did see some run-to-run differences, especially on cell growth. The Ambr250 platform has been shown to be more robust, and also, more representative of the production scale bioprocesses. **Despite the differences in scale and cell culture performance, our REBEL-data-derived amino acid consumption calculations were relevant and helpful across the scales.**

Our final scale-up experiment was performed in three 10L stirred-tank bioreactors (BR1, 2 and 3). The feeding strategy for all three bioreactors included pyramid feeding, every second day, with the CD feed plus the addition of amino acids in a separate bolus. The AA mix included essential amino acids only (as defined in the Ambr250 experiment). Calculations on

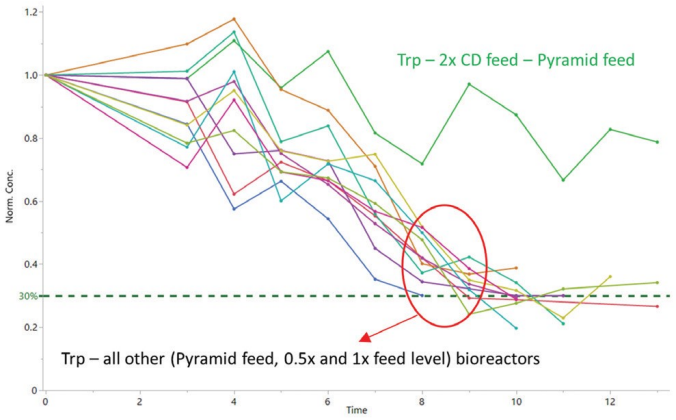

Figure 8. Comparison of tryptophan levels in bioreactors with pyramid feeding in Ambr250. Independent of the other amino acids added, in the low CD feed conditions tryptophan is depleting to or below 30% level. The only condition in which tryptophan is maintained is the control, high level of CD feed bioreactor.

Table 2. Feed strategy refinement step. Feeding volume (% v/v) in the every other day flat and pyramid feeding strategies for CD feed, starting either day 2 or day 3. The CD feed vendor-recommended concentration is "2x" with 4% v/v additions very other day.

Feeding day	2 or 3	4 or 5	6 or 7	8 or 9	10 or 11	12 or 13
CD Feed Pyramid feeding	2%	2%	4%	4%	2%	2% or 1%
CD Feed "0.5x" Flat feeding	4%	4%	4%	4%	2%	4% or 2%

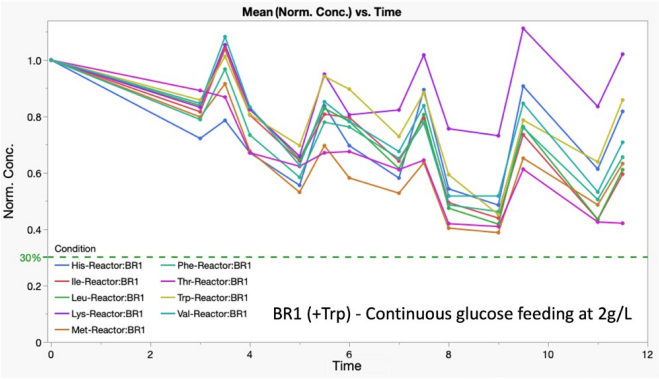

Figure 9A. Bioprocess scale-up. Essential amino acid profiles in BR1. The feeding strategy was optimized with a low CD feed level and an additional amino acid mix bolus feed containing only essential amino acid, including tryptophan. The concentrations are normalized to the starting concentration.

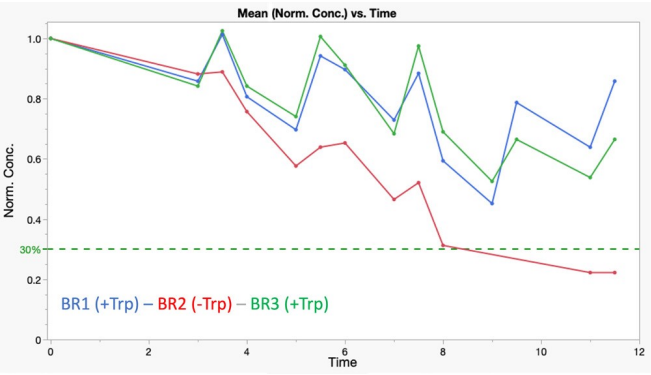

Figure 9B. Bioprocess scale-up. Tryptophan profiles in all three 10L bioreactors. In the control bioreactor, where tryptophan was not added (BR2), the level of tryptophan drops below 30% at day eight, when mAb production is ongoing. The concentrations are normalized to the starting concentration.

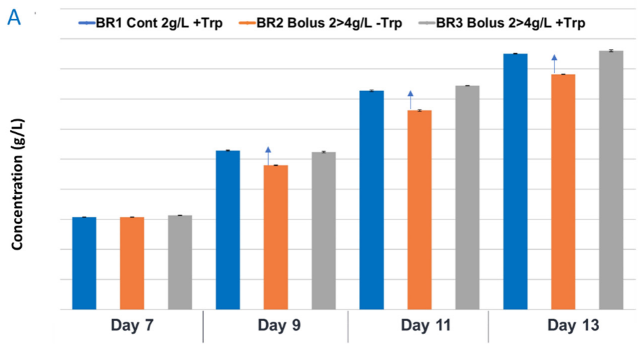

Figure 10A. Bioprocess scale-up. mAb titer from the final scale of 10L in the three different feeding strategies used (please see text for details). The addition of a single amino acid resulted in a slight (6%) increase in titer, whereas keeping the glucose levels lower, but stable, had no adverse effects on the production.

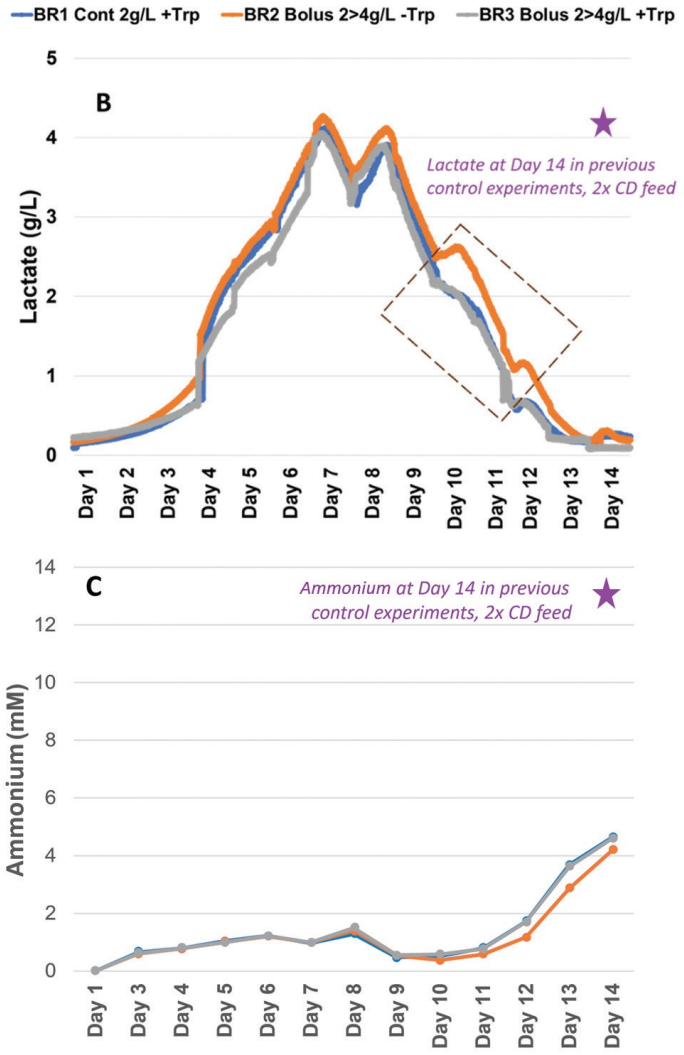

Figure 10B. Bioprocess scale-up. Lactate profile showed a shift between days 9–13 in BR2 (not supplemented with extra tryptophan). This shift happened after the tryptophan had dropped below 30% from the starting condition. Observing this shift was enabled by monitoring lactate levels real-time with MAVEN. 10 C) ammonium profiles from all three bioreactors are very similar. The purple star in the B) lactate and C) ammonium graphs at Day 14 indicate the level of these metabolites in previous experiments, vendor-recommended level of CD feed. The control condition of high level of CD feed was not included at this 10L scale.

how much to add were again based on daily REBEL analysis from the previous runs. From Ambr15 to Ambr250 the AA consumption rates were very similar (data not shown) and thus there was no need to adjust the AA concentrations.

In response to the tryptophan depletion in the previous experiment, we also tested the addition of extra tryptophan

into the amino acid mix in BR1 and BR3. BR2 was not supplemented with extra tryptophan.

From the amino acid trends throughout the time course of the bioprocess, we were able to see how amino acid supplementation mixes calculated from amino acid consumption were capable of avoiding depletion of essential amino acids (levels did not drop below 40% from the start concentration), but also, the levels fed did not cause accumulation (concentrations not above 110% of starting concentration). Figure 9A shows BR1 essential amino acids profiles in the time course.

Figure 9B shows the time course data for tryptophan in the three different conditions tested: two with additional tryptophan (BR1 and BR3), one without (BR2). In BR2 (no tryptophan) the level of this amino acid dropped below 30% on day 8 which coincides with the peak viable cell density (data not shown). This may have had an effect on the production, as the cells are actively producing the mAb product (see figure 9A). Furthermore, the titer was boosted by 6% (Figure 10A) by the addition of this single amino acid (BR3 compared to BR2), in concentration as determined by REBEL measurements of consumption.

At this scale, our focus turned to optimization of the glucose feeding strategy. Real-time monitoring of glucose and lactate was achieved using Repligen's MAVEN System, a biosensor-based on-line analyzer. One bioreactor (BR1) received continuous glucose feeding with a set-point of 2g/L, while the other bioreactors continued with bolus feeding. Please see the relevant application note for more details.[5]

Real-time monitoring revealed a shift in the lactate profile in the control bioreactor (BR2), where extra tryptophan was not added to the amino acid mix (see Figure 10B). This may indicate that the cells were shifting in some metabolic pathway to cope differently with the lower amount of an essential amino acid available. **Without real-time monitoring, this shift would most likely not have been noticed.**

These experiments showed our amino acid feeding strategy scaled-up well to the 10L scale. The tryptophan addition increased the titer by 6% (Figure 10A; BR3 compared to BR2). The continuous glucose feeding strategy, even when keeping the glucose at a relatively low level, did not have an adverse effect on the growth or production (BR1 compared to BR3).

The goals for the project were met at the final scale:

- We used a lower-than-recommended concentration of a commercially available feed to reduce the cost of goods and make the process more robust by improving the toxic metabolite profile (lactate and ammonium) in the bioprocess.
- Importantly, we had >60% viability of cells on day 14 of the fed-batch culture to reduce the risk of damage to the product in the late days of the process and simulate a more optimal starting point for the downstream process.
- Equally importantly, bioprocess product (mAb) quality was examined throughout our feed strategy optimization, refinement, and scale-up experiments by typical analytical methods, and no significant product quality differences were found in glycosylation or charge variant profiles.
- We were able to reach target titer despite using less feed than recommended, resulting in lower cost of goods.
- Additionally, the addition of single amino acid in the final 10L scale increased the titer by further 6%, as compared to the previously defined mix (all conditions low level CD feed).
- The feed strategy optimization including supplementation of specific essential amino acids scale from Ambr15 to Ambr250 and 10L bioreactors.

6. Data-driven process improvements resulting in cost savings

The 15 mL Ambr microbioreactors and 250 mL Ambr bioreactors were sampled in an automated fashion for the at-line and off-line analytical devices used to measure cell growth and other metabolites and nutrients, such as glucose, lactate, ammonium, etc. Adding a large amount of data points from REBEL spent media analysis did not increase the volumes required, or frequency of sampling, as the device only uses a very small amount (~50 µL) of a sample.

The data-driven approach of this project was enabled by REBEL. Frequent measurements of the amino acids in the spent media helped accurately calculate the feed AA mix addition concentrations to keep cell culture metabolites and thus cell culture conditions at desired levels. REBEL analysis time per sample is about 10 minutes. As compared to sending samples to an external lab where turn around times can measure in weeks or months, the sample analysis times are greatly reduced. The samples from an extensive run such as the Ambr15 experiment can be run at the bioprocessing lab bench, and thus the time for shipping, more extensive sample preparation and waiting for analytical results to come back, are all omitted. The cost per sample of shipping samples to an internal core facility lab or to an external can be estimated to be about $80 to $600 USD, respectively, making frequent amino acid measurements unrealistic for a project of this extent (multiple 14-day Ambr15 runs with 48 bioreactors). The performance and usability of REBEL was recently assessed by FDA contributors.[4]

From the cell culture perspective, an additional cost savings arise from the somewhat reduced cost of cell culture feed media. Calculate your savings using the cost savings tool: https://repligen.convertcalculator.com/calculatesavingswithrebelxt

Conclusions

By using a combination of analytical tools including Repligen's REBEL and MAVEN, we were able to gain unique insights that allowed us to perform a data-driven optimization of a bioprocess and reach the process development goals with regards to titer, toxic metabolite profiles, and viability.

Fast and simple REBEL analysis along with the Ambr15 and Ambr250 cell culture systems with multiplexing capability were considered essential for this work.

Multiplexing experiments enabled us to collect the relevant data far easier than separate bioprocess experiments could. Using REBEL as a spent media analyzer is feasible at all scales due to the low sample volume requirements and the fast analysis time for a comprehensive panel of analytes. The data was smoothly handled in JMP statistical software with JMP add-in for visualization. The amount of data produced from the experiments (titer, cell growth, nutrient, metabolite profiles, pH, gases, etc.) is large, and the visualization of the data takes time. We also used Sartorius UMetrics suite of software, MODDE and SIMCA, to guide data analysis.

MAVEN was utilized only at the last stage of the project and scale-up, but it proved to be an essential addition: **MAVEN monitoring saved two of the bioreactors from crashing in our last stage of the of the project.** Please see more information in relevant application note.[5]

The scale-up from Ambr15 to Ambr250 to 10L successfully exhibited the same key nutrient consumption profiles, **indicating that the REBEL analysis at early-stage PD can give invaluable insights that translate to scaled-up processes.** In addition to the data analysis already done, an interesting approach would be to look at cell-specific nutrient consumption rates and protein production rates at different phases of the cell culture. Nutrient consumption rates are expected to vary between the different phases (exponential growth, production, cell decline) and could provide essential information for further intensification of the process. These, and other types of insightful calculations are possible with the data from the daily measurements with REBEL. REBEL provides the concentrations of analytes reported in mM, and not just arbitrary monitoring of upward or downward trends of analyte levels.

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