Empowering process optimization for a mAb-expressing CHO cell line: Rapid at-line amino acid monitoring coupled with integrated multivariate analytics

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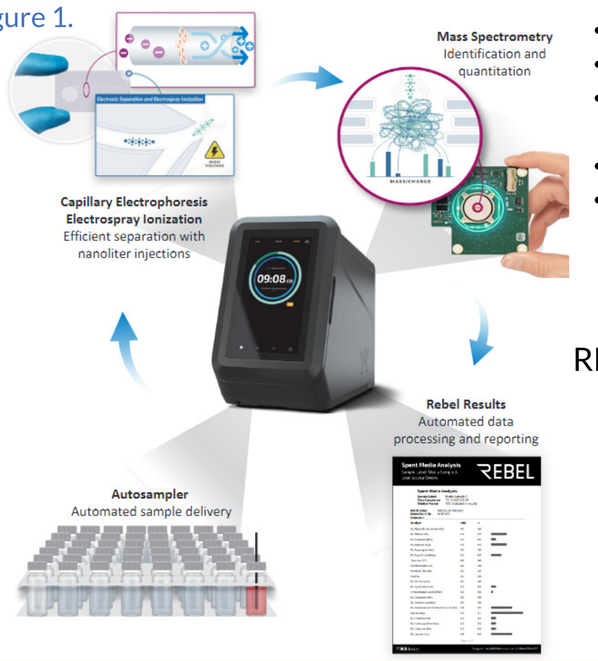
Overview

Optimization of cell culture media and process conditions are required for achieving optimal cell growth, productivity, and product quality. This is often hampered by the lack of point-of-need access to key nutrient composition and consumption in process development. Coupling miniature mass spectrometry (MS) with microchip capillary electrophoresis (CE) fulfills an unmet need: simple, rapid at-line nutrient quantitation. This case study exemplifies support of bioprocess optimization in an ongoing project with three Ambr 15 runs followed by scale-up to 10 L bioreactor complemented by DOE and multivariate analysis using Umetrics® tools.

Fresh and spent media analyzer

Figure 1.



- Minimal sample requirement as low as 10 µL
- Simple sample prep: spin or filter and dilute
- Integrated analyzer includes autosampler, separation, detection, analysis and reporting
- Analysis run-time ~10 min per sample
- Consumable kit optimized for 200 analyses

REBEL Spent Media Analysis Kit

Experimental design for bioprocess and feeding strategy optimization

1 Fresh Media Analysis	2 Media Screening	3 Media /Feed Optimization	4 Feed Strategy Refinement	5 Scale up
Amino Acid (AA) profiling in CHO media to select media for screening • Analyzed AA content of 8 CD CHO media panel	• 1st Ambr 15, fed-batch run screening 8 CD CHO media, one feed, two glucose levels • Measured metabolites and **daily amino acid consumption**	• 2nd Ambr 15, fed-batch run, 4 media/1 feed • **DOE:** 3 levels of feed (low, medium, high) and 4 levels of AA supplementation (0, low, medium, high)	• 3rd Ambr 15, fed-batch run, 1 media/1 feed • Inform: - Modeling - Final feed strategy - Scale up	• Ambr 250 and 10L bioreactors

Materials and Methods

Using a Sartorius Ambr 15 automated microbioreactor system, CPI's stable GS-mAb expressing CHO cell line was screened against a panel of 8 commercial chemically defined CHO media (Thermo Fisher Scientific) in triplicate bioreactors. Cells were cultured using a 14-day fed-batch process and a commercial feed medium. Based on performance and nutrient depletion, four cell culture media were selected further with different feed and amino acid (AA) feeding strategies.
Spent media AA analysis was performed daily using the REBEL analyzer. Spent media samples were filtered, then diluted 200x using REBEL diluent. Other spent media analysis were performed on the Beckman Coulter Vi-CELL XR, Roche CustomBiotech Cedex Bio HT, and Advanced Instruments 3320 Osmometer. Titer analysis was performed using the ForteBio Octet. Data was further processed with Sartorius Stedim Data Analytics AB Umetrics® tools - MODDE and SIMCA. This poster specifically highlights MODDE Design of Experiments (DOE) results.

Fresh Media Panel Analysis

Figure 2.

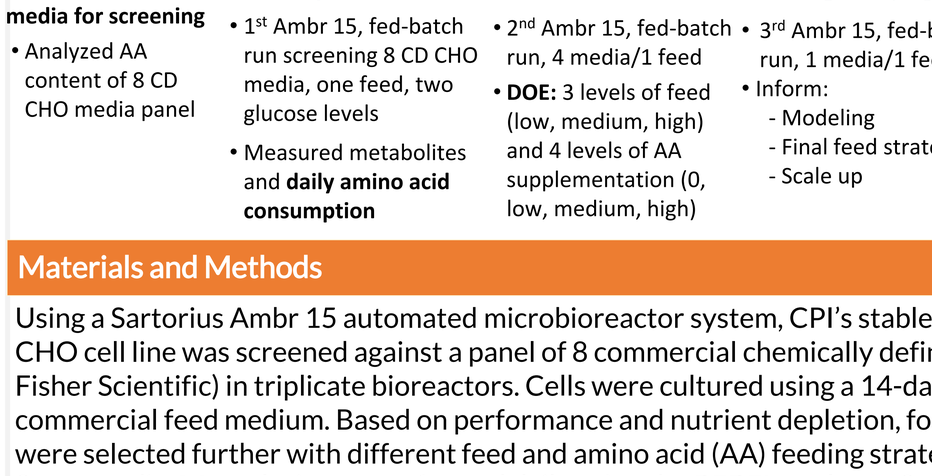


Figure 2
- *8 different CHO media were screened fresh to assess their composition*
- *AA concentrations varied across the media by 2 to 23-fold. Some formulations did not contain all AAs.*

First Ambr 15 run – Media screening

- Across the first Ambr 15 14-day fed-batch process, Medium 6 reached the highest viable cell count (Fig 3A) and IgG titer production (Fig 3B), Medium 9 the lowest.
- Despite a 2x difference in titer production between Media 6 and 9 (Fig 3B), there was very little difference in the glucose consumption, and low ammonia build-up across the cultures (Fig 3A).
- Medium 9 did not go through the lactate (Fig 3A) switch and exhibited high lactate production, and the lowest titer (Fig 3B) compared to the other media.

First Ambr 15 run - metabolite and titer analysis

Figure 3A.

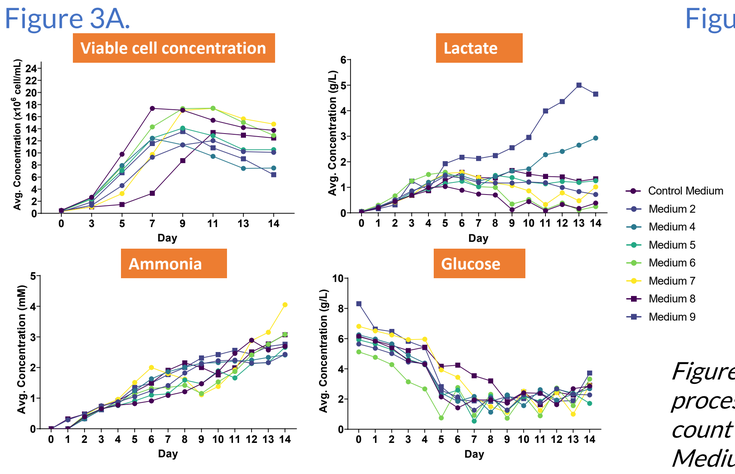


Figure 3B.

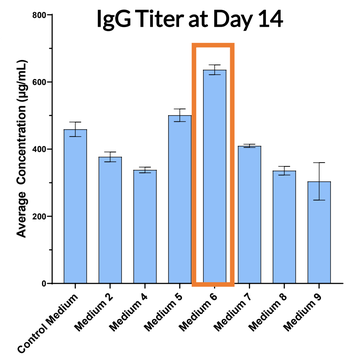


Figure 3. Across the first Ambr 15 14-day fed-batch process, Medium 6 reached the highest viable cell count (Fig. 3.A) and IgG titer production (Fig 3.B), Medium 9 the lowest.

First Ambr 15 run - Amino Acid Analysis (AAA)

Figure 4.

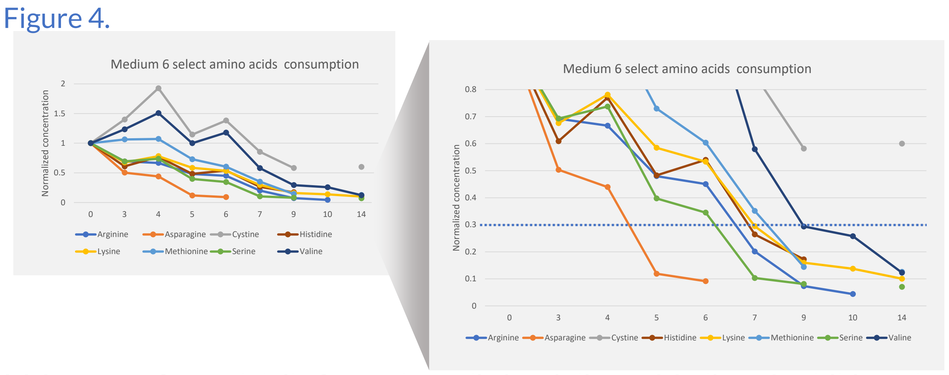


Figure 4. First Ambr 15 run showing the REBEL data for select amino acid consumption (normalized to time 0).

AAA was performed daily for each media in triplicate. Medium 6 exhibited depletion of many AAs falling below 30% of starting value (Fig 4), whereas in Medium 9, only Asn was depleted (not shown). The subset of AAs showing most significant depletion were selected for a custom AA mixture – an additional bolus feeding every other day for the second Ambr 15 run.

Second Ambr 15 run – amino acids added to feeding strategy

In the second Ambr 15 14-day fed-batch process, the feeding was changed to test the effect of added AA mix (Arg, Asn, His, Lys, Met, Ser, Val) at four levels : No AA, Low, Med, and High AA, in addition to 3 levels of feed: Low (as used in the first run), Medium, and High. Thus, each of four media (4, 5, 6, and 9) were tested in 12 vessels with combination of feed and AA mix levels.

Figure 5A.

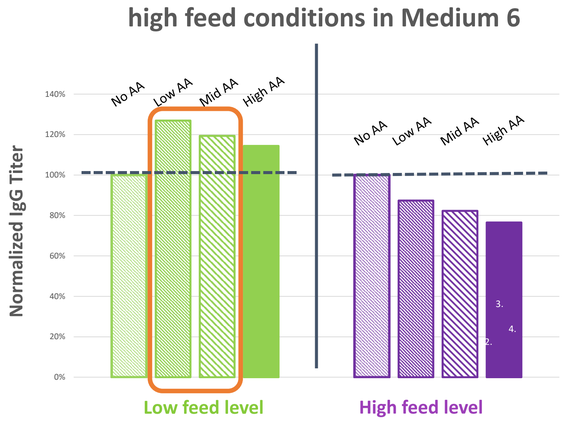


Figure 5B.

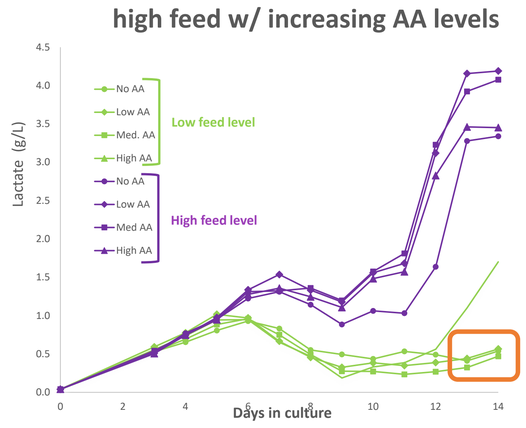
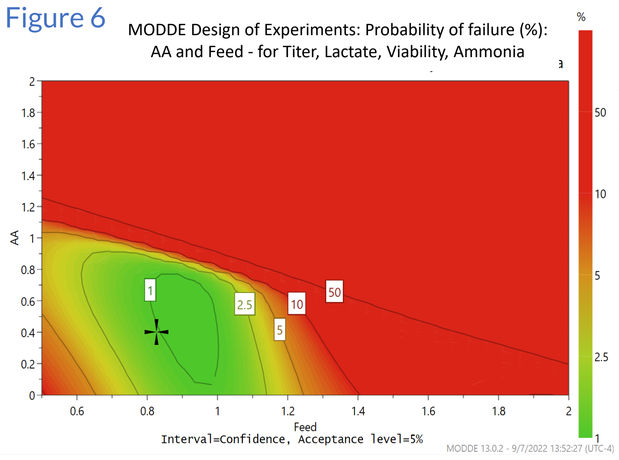


Figure 5
- *The addition of AA was effective with low feed (green Fig 5) achieving up to 38% improvement in titer yield in some media when compared to no additional AA feed. Results for Medium 6 (Fig 5A) show 27% improvement of titer (highlighted in orange).*
- *With higher levels of feed (medium and high; purple in Fig 5), adding more AA separately did not improve titer (Fig 5A), but resulted in the undesirable accumulation of lactate (Fig 5B). However, keeping the feed low, also maintained low lactate levels (highlighted in orange).*

Sartorius MODDE DOE analysis

Figure 6



Data from the second Ambr 15 run was fed into the Umetrics® MODDE tool for DOE. The experiment space was defined as exploring AA mix addition level and the feed level to result in favorable viability, titer, lactate, and ammonia levels.

Figure 6 shows the optimal feedand AA supplement space in green, otherwise referred to as the DOE sweet spot with low probability of failure in the set criteria.

Conclusions and future work

The work demonstrated how low-level feeding strategy can be optimized for titer and lactate and ammonia profiles by adding AAs based on their consumption measured daily from 14-day fed-batch culture, rather than adding more of the commercial feed. Economical benefits are immediately recognized in resource savings, e.g. personnel, time, and cost of cell culture feed. Next, data shown here will be supplemented with product quality analysis. This data will be further analyzed with the Sartorius Umetrics® tools to optimize the next round of experiments in microbioreactors as well as in scale-up.