Data-driven feed strategy optimization and scale-up of mAb-expressing CHO cell line bioprocess

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Overview

Biologics production development for intensified processes aims to achieve desired product quality attributes and high productivity. Running multiple micro-bioreactors in parallel enables expedited optimization, but low microbioreactor volumes limit the media available for daily sampling for analysis. Cell culture media (CCM) optimization can be a lengthy and complex process involving multiple DOEs. Fast, at the point-of-need, analytical tools with only minimal sample consumption, are required to assess key-nutrient consumption and provide feedback to enable feed strategy optimization.

Fresh and spent media analyzer

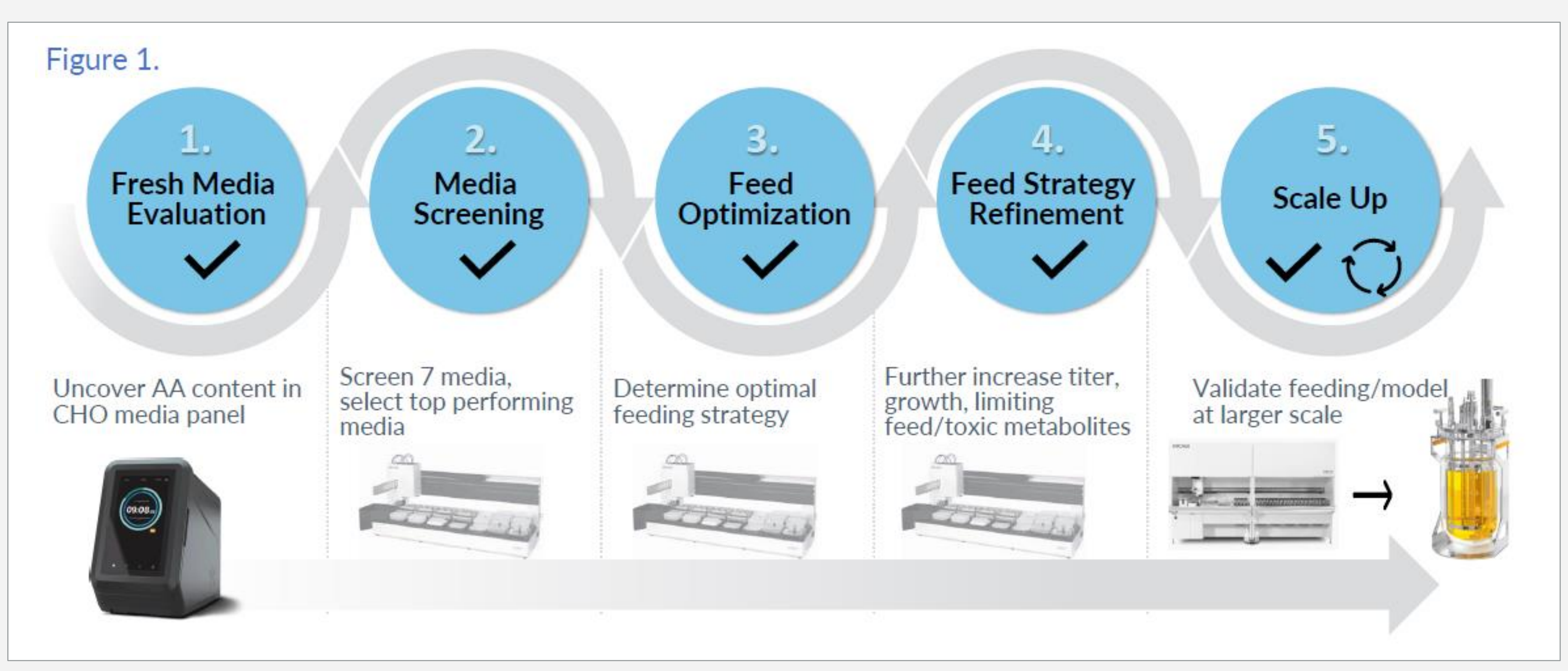
PATsmart™ REBEL® analyzer

Spent Media Analysis Kit

- Minimal sample requirement: as low as 30 µl
- Simple sample preparation: centrifugation or filtration and dilution
- Integrated analyzer includes autosampler, separation, detection, analysis and reporting components
- Analysis run-time ~10 min per sample
- Consumable kit optimized for analysis of 200 samples

Experimental design for bioprocess and feeding strategy optimization

Figure 1.



Materials and methods

- Sartorius Ambr15® and Ambr 250® automated microbioreactor systems
- 10 L bioreactors: Biostat 10 L stirred tank bioreactors (Sartorius Biostat B-DCU II with BioPAT DCU Tower)
- GS-mAb expressing CHO cell line, 14-day fed-batch process
- Commercial chemically defined CHO basal and feed media acquired from Thermo Fisher Scientific.
- Feed media was used in standard condition as per vendor recommendation, at 2x level (high).
- Customized feeding strategies were tested to control for toxic metabolites (lactate and ammonia) and maintain viabilities while increasing titer using the feed medium at medium (1x) or low (0.5x) levels with addition of different levels of groups of amino acid (AA) based on spent media analysis.
- Spent media amino analysis was performed daily using the REBEL analyzer: samples were filtered, then diluted 200x using REBEL diluent.
- Viable cell densities and viability were analyzed daily using Beckman Coulter Vi-CELL XR.
- Glucose, lactate, ammonia, LDH, Gln and Glu were analyzed daily using Roche Custom Biotech Cedex Bio HT, and titer analysis was performed using the Octet, Sartorius.
- Data was further processed with Sartorius Data Analytics AB Umetrics® tools—MODDE and SIMCA.

Ambr 15: Initial feed optimization using amino acid spent media

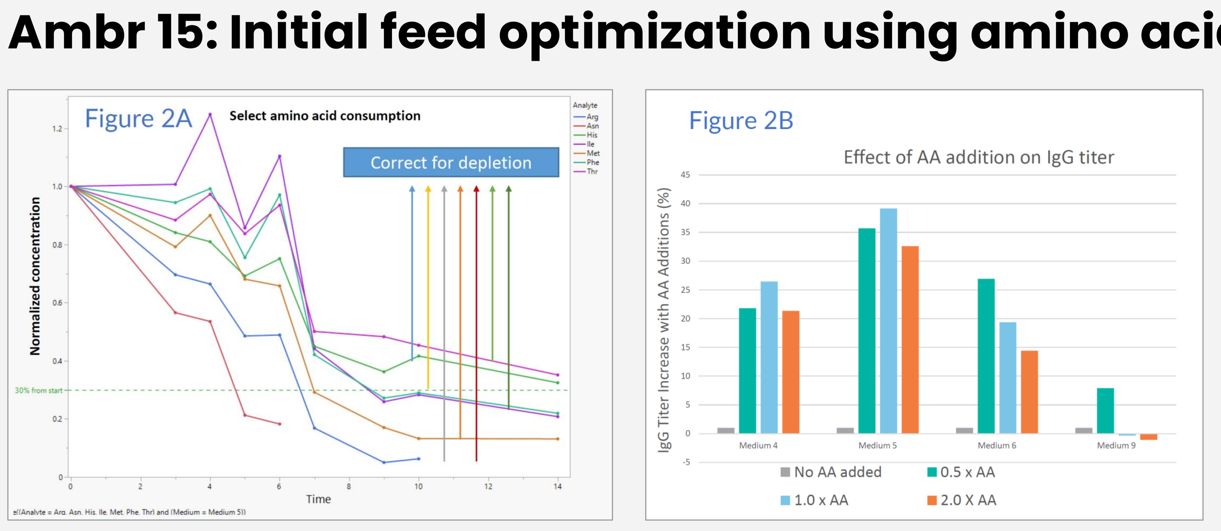


Figure 2. Development of a customized feeding strategy

A. Spent media analysis revealed the depletion of several amino acids during the fed batch time course. AA additions in bi-daily feeding was calculated from REBEL data to refill to starting concentration = 1.0 x AA

B. Addition of amino acids to remedy depletion results in up to 40% increase in titer, with 10% of standard feed (high feed) strategy

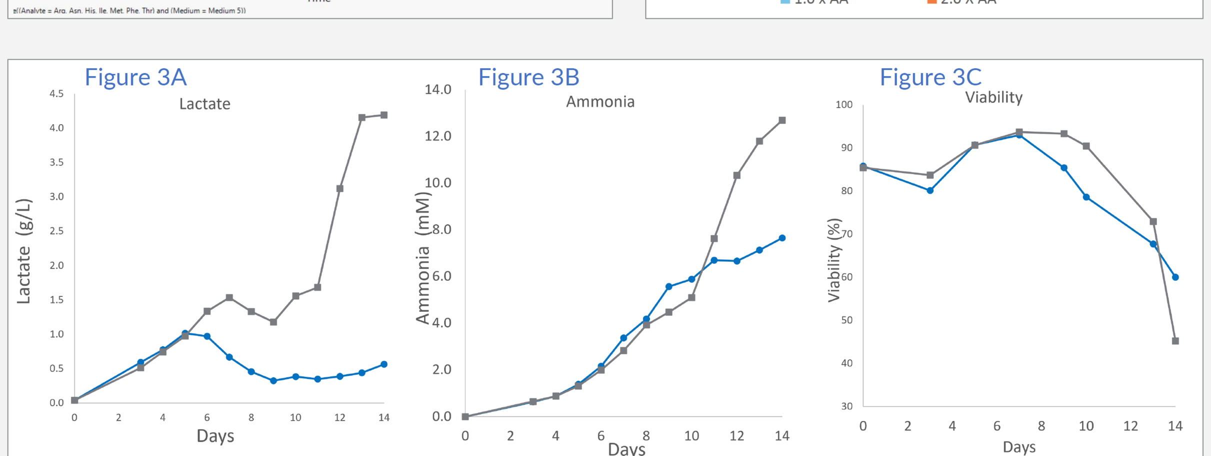


Figure 3. Lactate (**3A**) ammonia (**3B**) and viability (**3C**), and profiles in customized and standard feeding strategies.

Ambr 250: Feed strategy refinement and scale-up

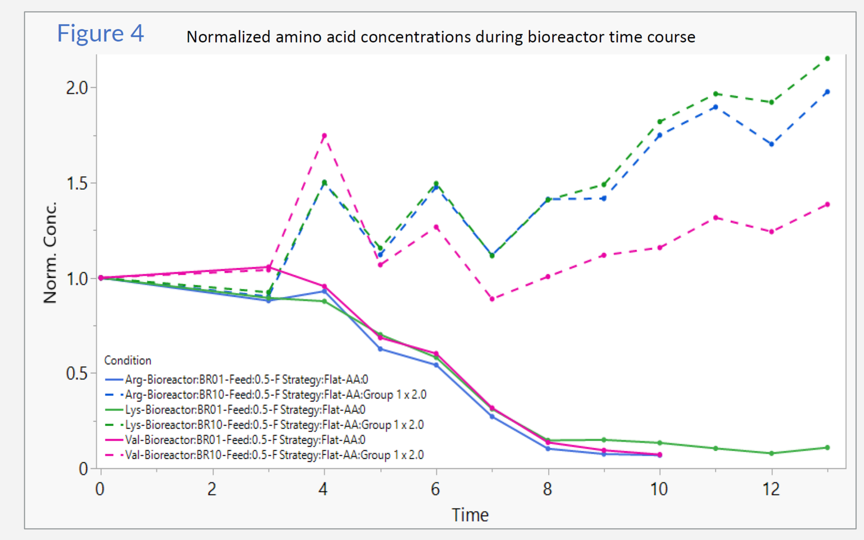
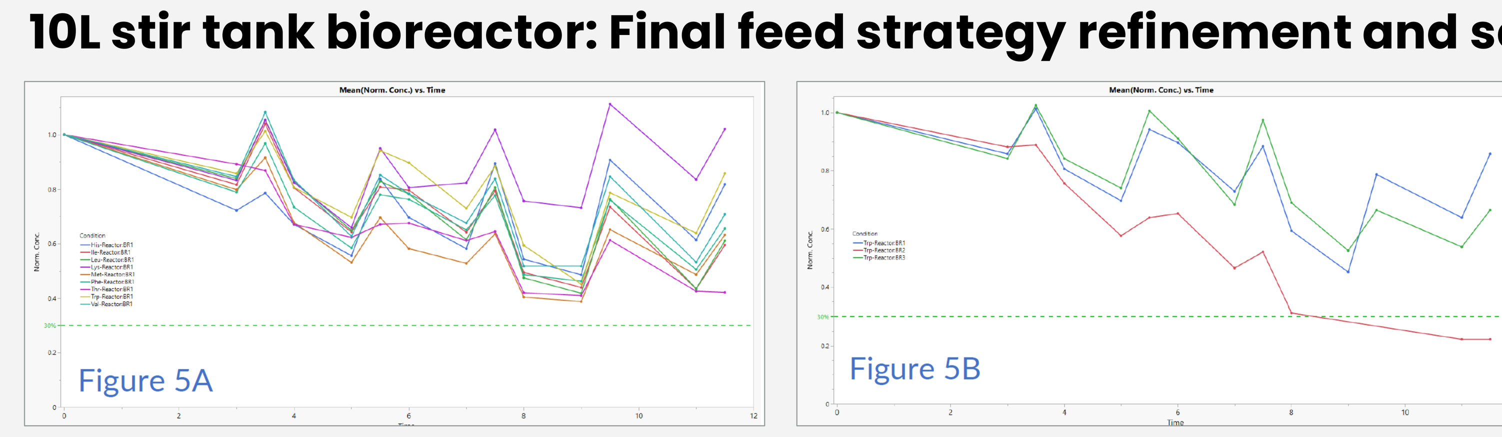


Figure 4. In Ambr250 run, standard feeding, several amino acids depleted below 30%, even as feed medium was added every other day starting on day 3. In customized feed, the complete feed was supplemented with amino acids based on depletion patterns from Ambr15 runs' REBEL analysis. This strategy kept the levels of amino acids from depleting, but in cases caused accumulation.

10L stir tank bioreactor: Final feed strategy refinement and scale-up



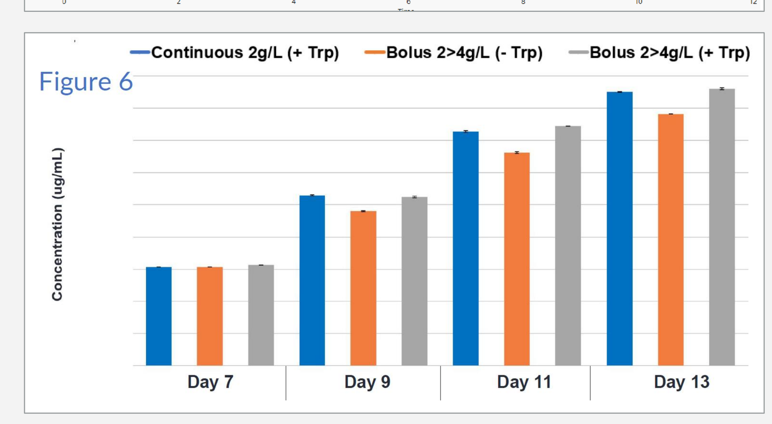


Figure 5. Essential amino acid profiles in BR 1 (5A; customized feeding) and tryptophan in all three 10L bioreactors (5B) One bioreactor (BR2) was not fed tryptophan, in which that aa depleted below 30% at day 8 – coinciding with peak VCD and production (not shown).

Figure 6. In the 10L scale all bioreactors received the previously determined customized feed, with the only exception of BR2 not being fed tryptophan in that mix. AA feed strategy based on REBEL information scaled up to 10 L.. Trp addition to EAA mix improved the titer by further 6 % (Day 13)

Real-time monitoring of glucose and lactate and glucose control

The PATsmart™ MAVEN® System was deployed at the 10 L scale: Automated feeding enabled by frequent on-line measurement of glucose can be used to create very consistent and stable glucose levels in cell culture. This strategy was used in BR1. Stable glucose levels may reduce the stress variable (alternating high-low levels of nutrient) conditions often occurring in bolus-feeding strategies, and may lead to improved cell growth and viability (cell culture longevity and reproducibility) as well as reduced metabolite profiles. Here, we saw no negative impact of continuous, low level glucose feed in bioprocess previously optimized for bolus feeding (BR2 and 3).

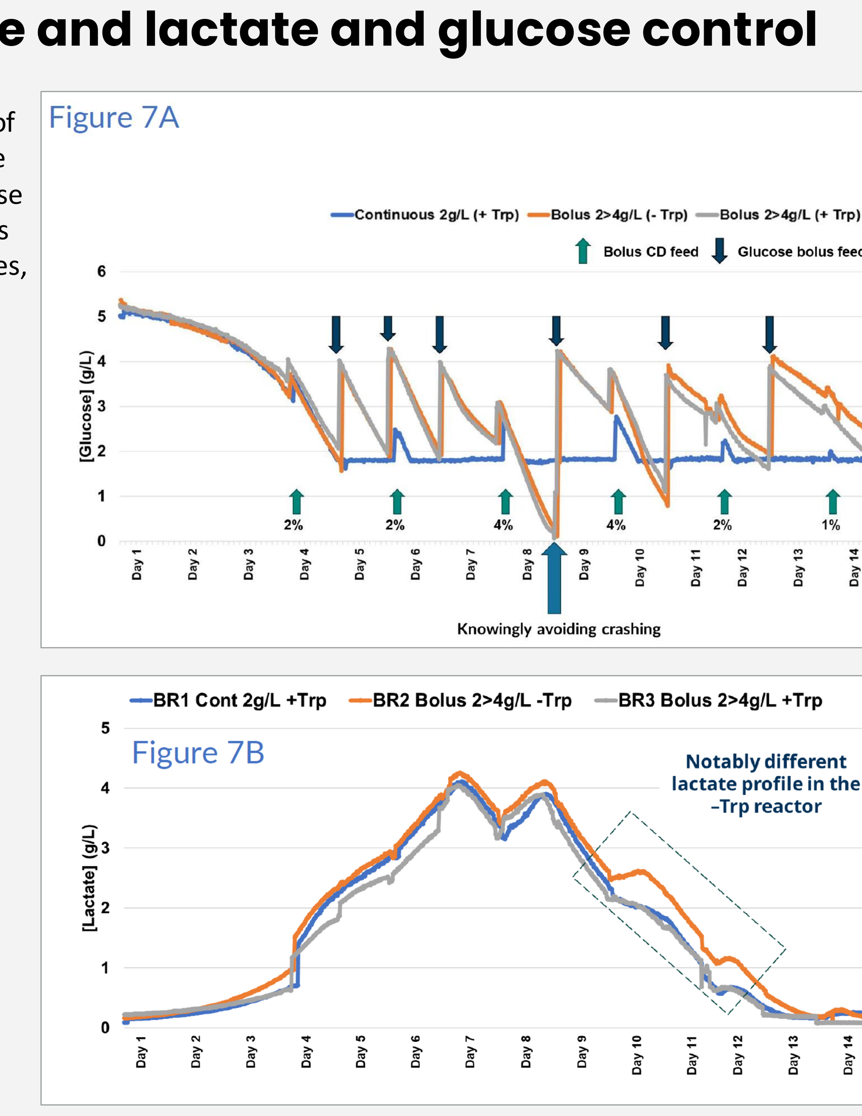


Figure 7A. Glucose measurements using the MAVEN in a CHO 10L bioreactor run: bioreactor 1 (continuous 2g/L glucose controlled by MAVEN), bioreactor 2 (bolus 2 to 4 g/L glucose (-Trp) and bioreactor 3 (bolus 2 to 4 g/L glucose (+Trp). On day 8 the glucose levels dropped close to zero in bioreactors 2 and 3. **A potential cell culture crash was avoided by monitoring the glucose levels closely with the MAVEN real-time measurements and adjustment of glucose feeding time.**

Figure 7B. Lactate profiles in run 1 for all three bioreactors. The real-time measurements showed a shift in the days 9-13 for the bioreactor that was not fed extra tryptophan in the amino acid mix.

Key findings

- Customized amino acid supplementation provided optimized process with low lactate, high viability.
- Essential AA feeding provided highest increase in titer without increase in lactate and ammonia
- Process was scaled from Ambr15 to Ambr250, and beyond to 10 L
- Continuous glucose and lactate monitoring and glucose control improved the cell culture operation and gave (real-time) insights on metabolic shifts.

Conclusions

- The work demonstrated how low-level feeding strategy can be optimized for titer and ammonia and lactate profiles by adding specific amino acids based on their consumption, rather than adding more of the commercial feed.
- The project goals were reached to keep titer level and improve cell culture conditions for more robust, and better controlled bioprocess.
- Automated on-line glucose monitoring was implemented at 10 L scale, which saved bioreactors from crashing

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