Amino Acid Quantitation of Cell Culture Media Matrices via an Integrated CE-MS Analyzer

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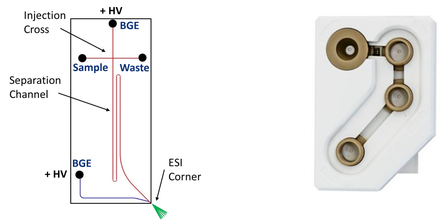
Overview

Optimization of cell culture media and process conditions are critical for achieving optimal cell growth, productivity, and product quality. Additionally, cell culture media contains many components that can complicate analysis via traditional analytical tools. The coupling of miniature mass spectrometry (MS) to a microchip capillary electrophoresis (CE) platform provides a simple solution for at-line amino acid quantitation.

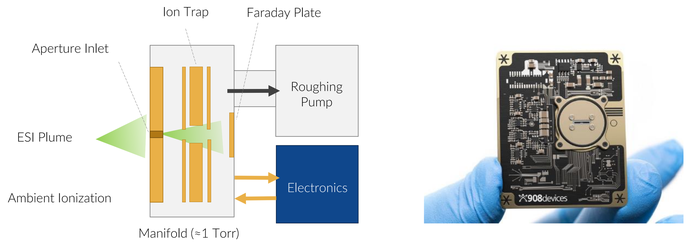
Core Technology

CE-ESI Chip



CE-ESI chip performs separation and generates positive mode ions on-chip

Ion Trap Mass Spectrometer



Ion trap mass spectrometer operates at ≈1 Torr, eliminating need for turbomolecular pumps

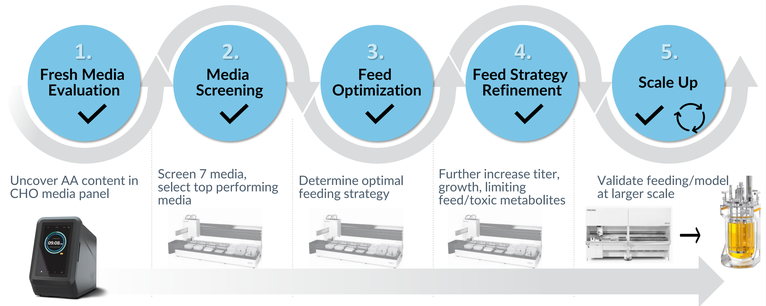
Integrated At-Line Amino Acid Analyzer

REBEL Analyzer

Spent Media Analysis Kit

- Integrated analyzer includes autosampler and all CE-MS components
- Consumable kit optimized for analysis of 200 samples
- Automated on-board algorithms compute concentrations, complete calibrations, and generate pass/fail reports

Bioprocessing Optimization



Feed Optimization

- Essential amino acid profiles in ambr250 bioreactors were measured across 12 days
- **Standard feeding:** feed medium was added every other day starting on day 3
- **Custom feeding:** complete feed was supplemented with amino acids based on observed depletion patterns

Feed Strategy Refinement

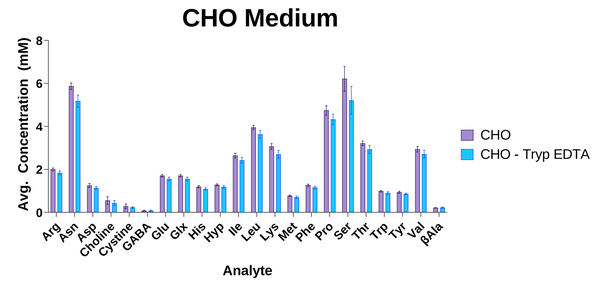
Titer increase in customized feed over standard feed condition

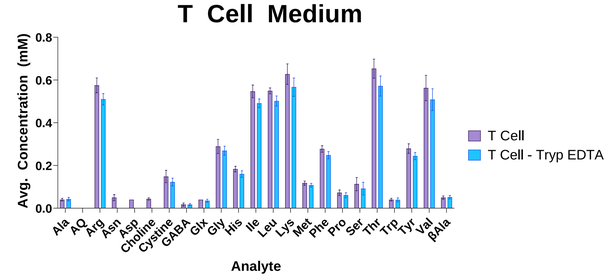
Bioreactor	BR6	BR8	BR9	BR10	BR11	BR12	BR13	BR14	BR17	BR18	BR19
Feed strategy	Flat	Pyr.	Flat	Flat	Pyr.	Pyr.	Pyr.	Flat	Pyr.	Pyr.	Flat
AA group	A	A	B	B	B	B	B	C	C	C	C
AA level	1	1	0.5	2	0.5	1	2	0.5	1	2	2

AA group	Amino acids in each group
A	Arg, Asn, His, Lys, Met, Ser, Val
B	Arg, His, Ile, Leu, Lys, Met, Phe, Ser, Tyr, Val
C	His, Ile, Leu, Lys, Met, Phe, Val

- Subsets of the depleted amino acids were fed to determine which were critical to titer production
- Customized feeding strategy resulted in up to 25% increase in titer
- The pyramid-based strategy was beneficial for titer increase in several conditions

Trypsin EDTA Spiked Media





- Three different media types were spiked with 10x Trypsin EDTA solution
- For each media type, a neat sample and a Trypsin EDTA spiked sample were prepared
- Using REBEL diluent, CHO media samples were diluted 100x, insect media samples were diluted 200x, and T cell media samples were diluted 25x
- Quantitative results were obtained by analyzing the samples on a REBEL system with n=12 replicates (3 vials, 4 reps per vial)
- 90% of the results had <20% difference between the basal media and spiked media samples. Within the calibrated range, 94% had <15% difference.

Literature References

1. K. Elliott et al., *BioProcess Journal*, 2020, 19
2. Y. Chen et al., *Biotechnology and Bioengineering*, 2022, 119, 435-451
3. K.H. Blakeman and S.E. Miller, *Portable Spectroscopy. and Spectrometry 1. Technologies and Instrumentation,* 391-413, 2021
4. M.J. Aernecke et al., *Sensors and Actuator Reports*, 2022, 4, 1000076