

A Rapid Microfluidic Method for Molecular Weight Determination and Spent Media Analysis of an IgG1 Intact Protein in Growth Media

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Overview

The analysis of cell culture media is a daily practice during the optimization of bioreactor conditions and media composition for the process development of biotherapeutics. The introduction of parallel microbioreactor systems has decreased the complexity and costs of process development by allowing for concurrent studies of multiple bioreactors and media variables; however, the bioreactors' small volumes (typically less than 250 mL) limit the quantity of media one can extract for daily sampling. In this study, we present a simple multi-level characterization approach for intact, reduced and subunit analysis of proteins in growth media using microfluidic CE-MS and correlate the findings from spent media analysis performed on an integrated analyzer.

Methods

Cell culture

Ammonia (NH₃) stressed bioreactor cultures of recombinant CHO-K1 cells were grown using an Ambr® 250 high throughput bioreactor platform (Sartorius) as described elsewhere (BioProcess J, 2020; 19. <https://doi.org/10.12665/J19OA.Elliott>). Samples were derived from time points taken over the course of a 14-day growth cycle.



Spent media analysis

The bioreactor samples were centrifuged at 9000xg for 15 mins to remove cells. The supernatant was diluted 1:100 with analyzer diluent and run directly on a PATsmart™ REBEL® analyzer (Repligen). Additional metabolites and cell analytics were acquired with a Cedex Bio (Roche) and a ViCell (Beckman), respectively.



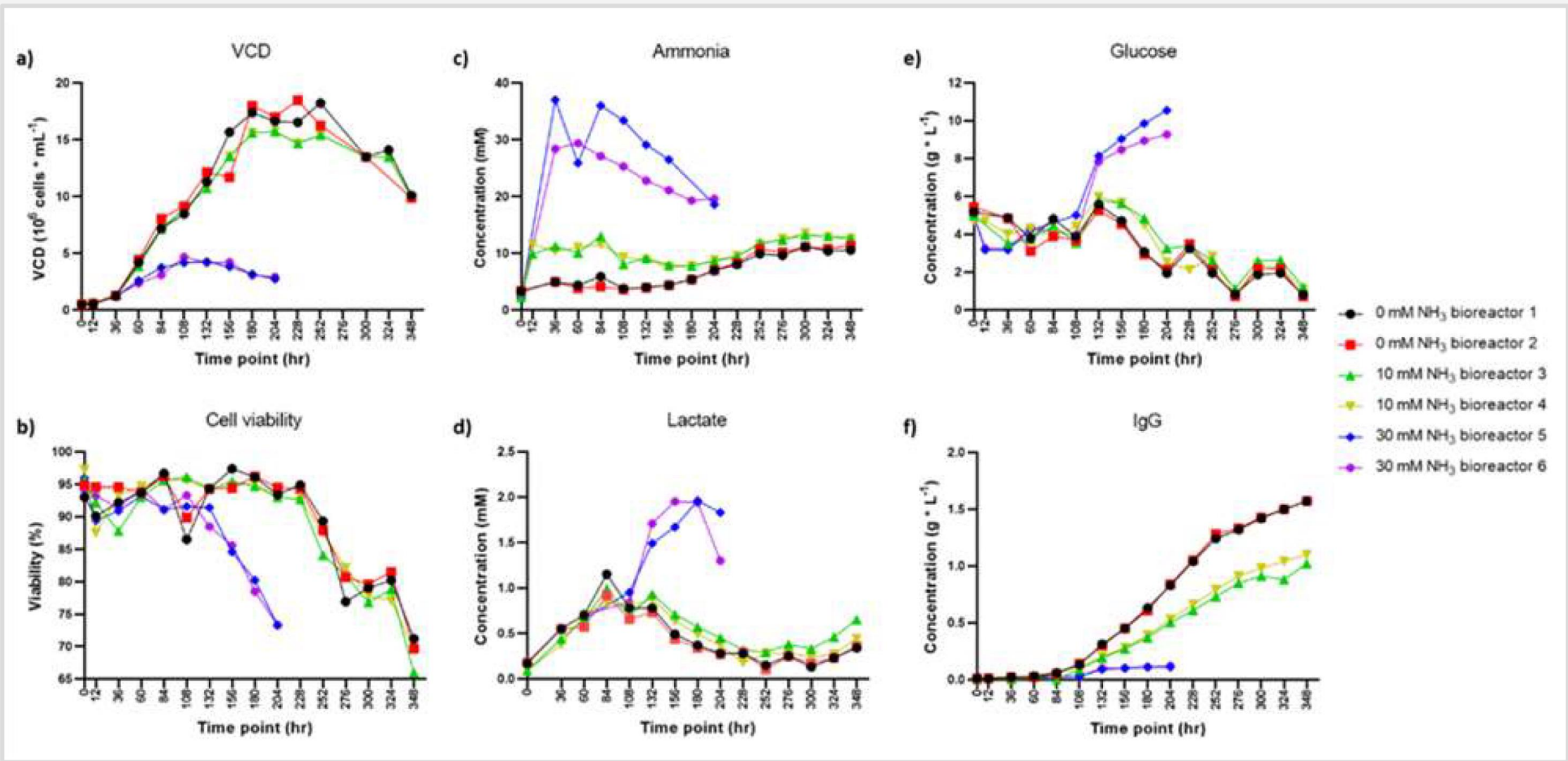
Intact and middle up analysis

For intact mass analysis, after centrifugation, bioreactor samples were directly loaded into the microchip and analyzed without any sample preparation. For reduced and subunit analyses, samples were buffer exchanged into Native Antibody BGE (Repligen) using Zeba™ Spin Desalting Columns, 40 MWCO (Thermo Scientific). Buffer exchanged mAb was subjected to proteolysis using the IDEs enzyme and reducing them with DTT for generating subunits. For reduced analysis, buffer exchanged sample was reduced by treatment with DTT.

All intact and middle-up analysis was performed using a microfluidic CE-ESI system (PATsmart™ ZipChip® System, Repligen) with a 22 cm long separation channel chip (ZipChip HR). Data were collected on a Thermo Q-Exactive HF. For intact mass analysis, the ZipChip Peptides BGE was used and 1.5 nL sample was injected. Reduced and subunit samples were analyzed using the ZipChip Metabolites BGE with 1 nL sample injected.

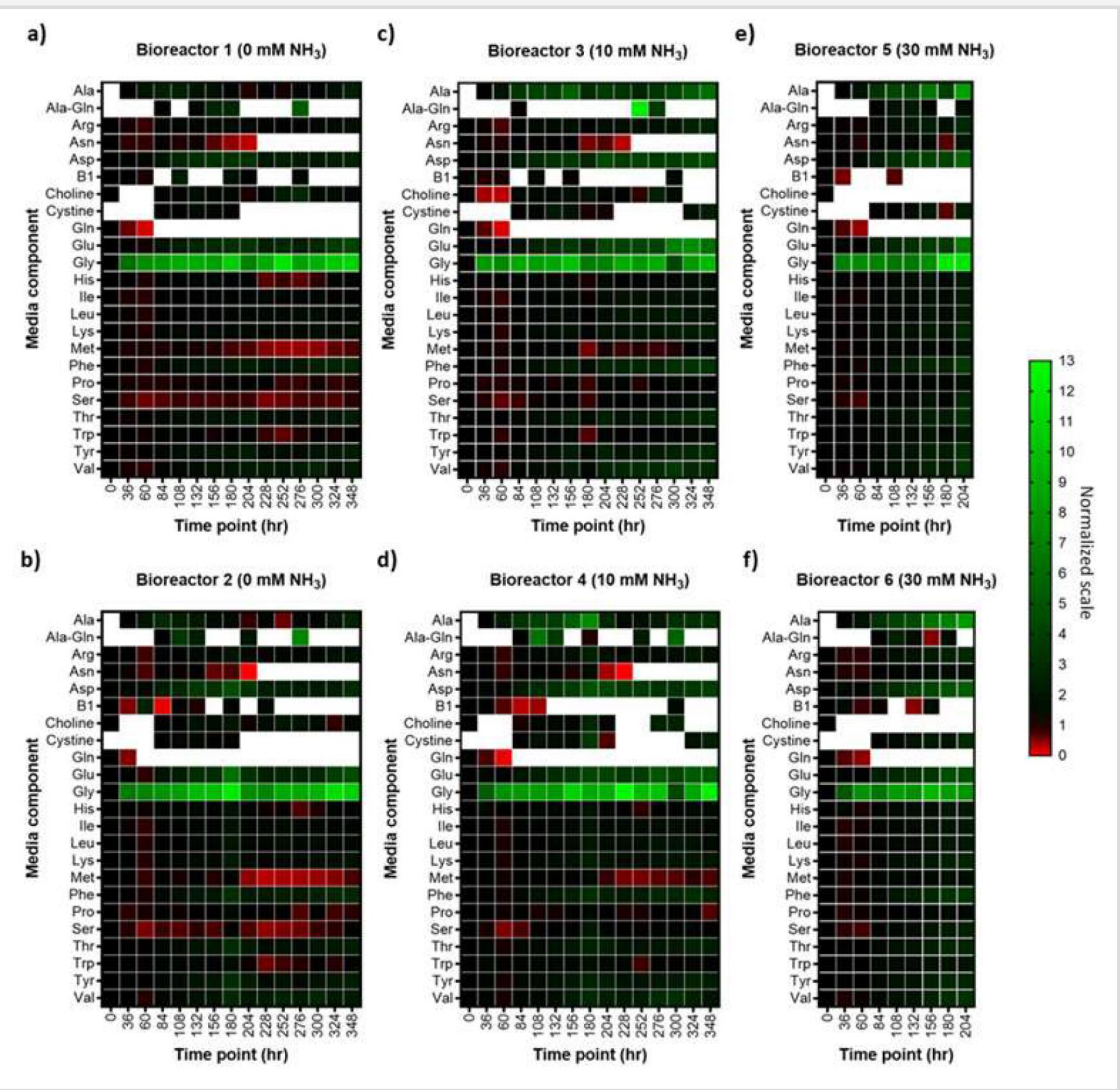


Spent Media Analysis



Daily biochemical analyzer data

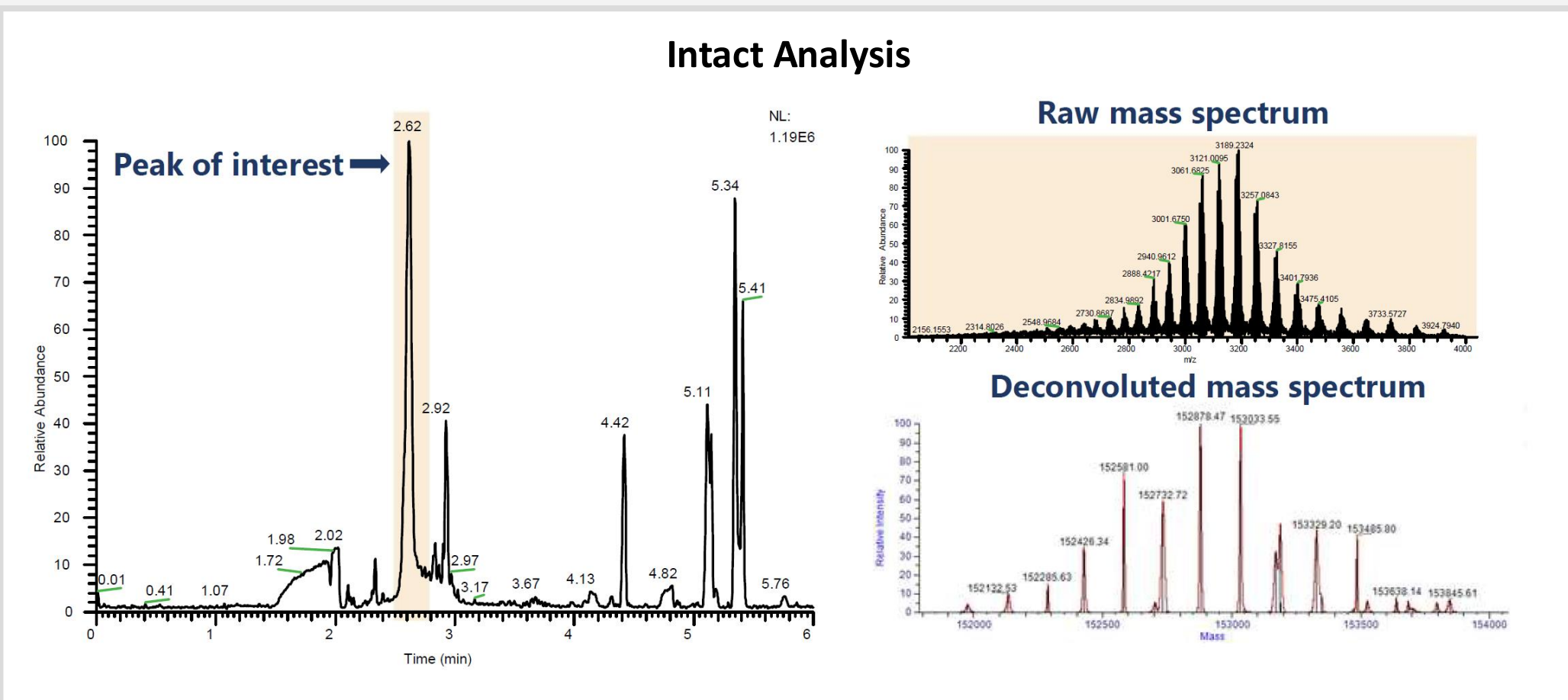
Cellular and biochemical profiles for duplicate unstressed (0 mM), 10 mM and 30 mM NH₃-stressed bioreactors: a) viable cell density (VCD), b) cell viability, c) NH₃, d) lactate, e) glucose and f) IgG protein (titer) concentrations. Approximately 1.4x more IgG produced in the unstressed system compared to the 10 mM NH₃-stressed bioreactors and 13x more IgG produced in the unstressed system compared to the 30 mM NH₃-stressed bioreactors. VCDs, cell viability, lactate and glucose values between the unstressed and 10 mM NH₃-stressed bioreactors were very similar throughout the process. There were more pronounced changes in cell viability, lactate and glucose levels for the 30 mM NH₃-stressed bioreactors after approximately 132 h into the process.



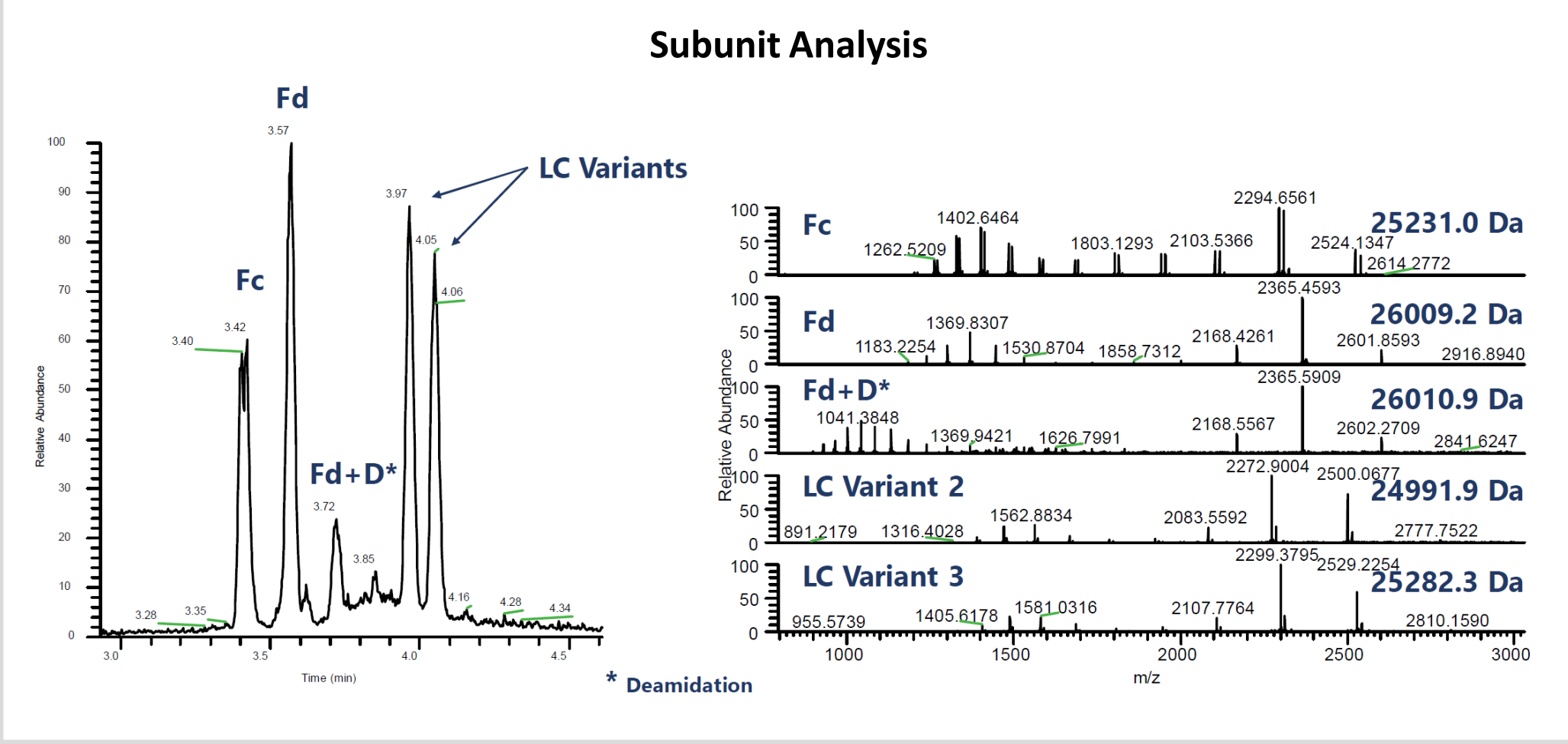
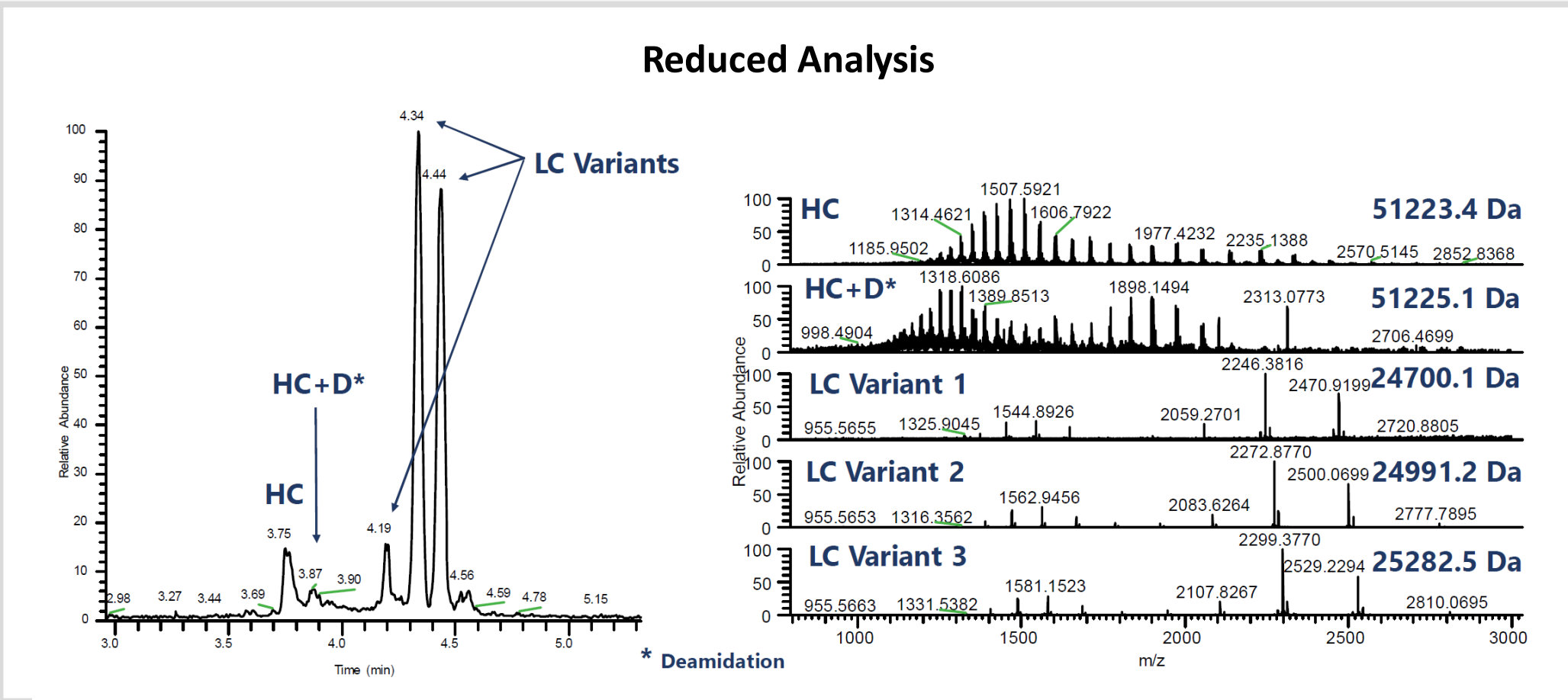
Cell media flux measurements

Normalized spent media analyte concentrations from the six bioreactors. Normalized values are relative to the first time point of detection of each analyte for the 0 mM (a and d), 10 mM (b and e), and 30 mM (c and f) NH₃-stressed bioreactors. The levels of the essential and non-essential amino acids changed independently and earlier in the process. Due to NH₃ stress, AA flux changes were more sensitive than the standard metabolite panel (glucose, lactate and Gln). In order to fully elucidate the mechanisms behind these amino acid flux changes and the relationship to protein production and quality, more frequent sampling is suggested.

Intact and Middle-up Analysis



The peak of interest for the intact IgG1 had a migration time of 2.6 min. The deconvoluted spectrum for the IgG1 appeared to be heterogeneous and showed several proteoforms with a delta mass that was not consistent with glycosylation.



Extended characterization using a middle-up approach was used to probe the heterogeneities. Separate reduced and subunit analyses were performed with excellent efficiency in <5 min. The same three species were observed in both reduced and subunit analyses confirmed their identity as light chain species. Also, a peak consistent with the glycosylated heavy chain was observed in reduced analysis.

Conclusions

Critical process parameter analysis of spent media with the Rebel and CQA monitoring for product characterization with ZipChip-MS provided a turn-key approach for rapid multi-level characterization of bioprocess samples. Fast analysis times (5-10 mins) coupled with minimal sample volume requirements (<10 µL) and sample preparation makes these microfluidic approaches a solution for high throughput process development groups.



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