In-depth metabolic characterization at different phases of cell culture with a turnkey CE-ESI-HRMS workflow

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Introduction

Understanding of cell culture metabolism in biologics manufacturing has increased tremendously in the past decade; however, the lack of feasible methods of high coverage, quantitative information on the interplay between nutrients and metabolites has hindered the efforts to fully characterize processes in the different biological phases. Several published models support cell growth phase but lack the kinetic information to be applicable to the phases that follow. We discuss a method for measuring hundreds of metabolites in dozens of pathways that streamlines sample preparation from spent media and cells, enables fast data-analysis times and supports quantitative analysis of key metabolites to gain far richer and more reliable data sources for process characterization, modelling and understanding. The method uses microchip CEMS and incorporates stable isotope internal standards for both quantitation and migration time indexing. This work explores the information that can be obtained from the extracellular growth media and intracellular metabolites extracted from the CHO cells.

Materials and Methods

Bioreactor Setup

Two bioreactors (BIOne 1250, Distek) were run with NISTCHO cell lines expressing NIST mAb. One received bolus feeds of glucose (<4 g/L bring to 6 g/L) while the other was fed continuously with glucose to maintain ~2 g/L glucose. Glucose, lactate, and viable cell density were measured using a Nova Flex 2 (Nova Biomedical). Samples of media and cells were removed ~2x daily and stored at -80° C until analysis.

Charge Variant Analysis

Charge variant analysis was performed using the Charge Variant Analysis Kit (Repligen) and a PATsmart™ ZipChip® interface coupled to an Exploris 240 Biopharma MS (Thermo Scientific). Data was processed using Biopharma Finder 5.0 (Thermo Scientific).

Metabolite Extraction and Analysis

Sample preparation was performed using the MoveKit (Move Analytical). Spent media was diluted 10x with PBS. Metabolites were extracted using the following method:

- Protein precipitation via methanol containing internal standards
- Addition of aqueous ammonium acetate solution
- Filtration to remove particulates

Cell pellets were washed with PBS and lysed using a methanol/ammonium acetate solution and sonication. Particulates were removed via centrifugation through a glass fiber filter plate.

Experimental setup was accomplished via the MoveKit app (Move Analytical) with data collection performed on an Exploris 240 Biopharma mass spectrometer (Thermo Fisher Scientific). Raw files were processed through Skyline (University of Washington) with additional peak assignments applied through the ACE App. Statistical analyses and trend plots were generated using Excel (Microsoft) and JMP 17.0.0 (SAS Institute).

MoveKit Consumables   **ZipChip HR+Orbitrap MS Data Acquisition**   **Automated Data Processing**

Results

Figure 1 shows several at-line measurements of the bioreactors monitoring key parameters, such as viable cell density and titer. 152 media metabolites and 240 intracellular metabolites were detected from the ACE panel. Many measured metabolites, such as canonical amino acids, displayed expected trends for a fed-batch CHO culture. By obtaining data from both the media and cells in the bioreactor, the interplay between media metabolites and intracellular processes can be assessed. Many measured metabolites, such as canonical amino acids, displayed expected trends for a fed-batch CHO culture.
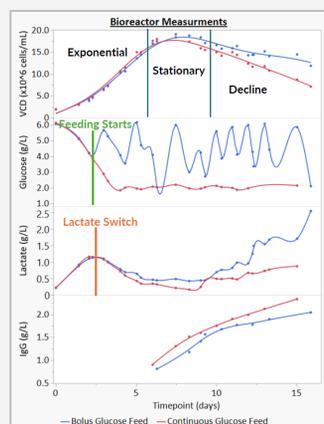

Figure 1. (left) At line measurements of the bioreactors. Although VCD is lower for the continuously fed bioreactor, titer levels are higher indicating a more productive culture.

Figure 2. (right) Trends of canonical amino acid consumption. The trend plots appeared as expected for these analytes from both bioreactors. Shown are the results from the Bolus Glucose Feed bioreactor.
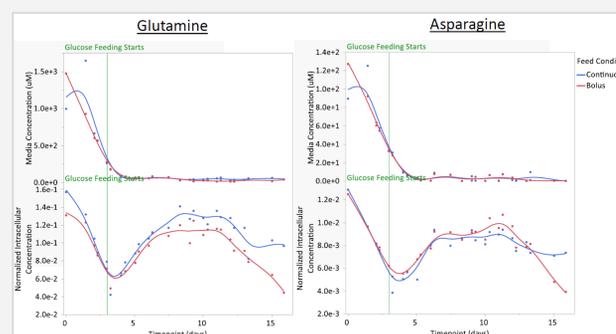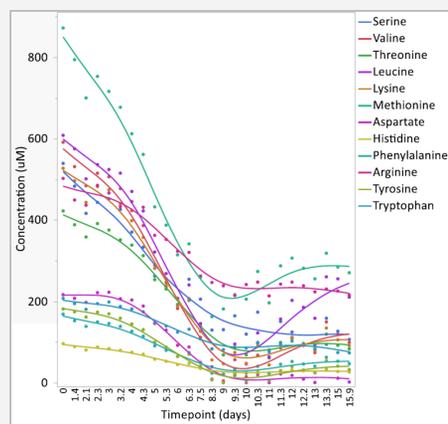

Figure 3. (above) Media and intracellular measurements of glutamine and asparagine, both sources of carbon in addition to glucose. Both bioreactors show similar trends with consumption of media glutamine and asparagine by Day 5. Intracellular levels increase through the stationary phase and begin to decline after ~ Day 12.

To highlight metabolic differences at different phases of growth, a pairwise t-test was performed using all data points within a growth phase. Many analytes measured showed significantly different trends at either the entry to the stationary phase or as culture progressed toward to decline phase. Below are trend plots for select analytes with p values <0.05.
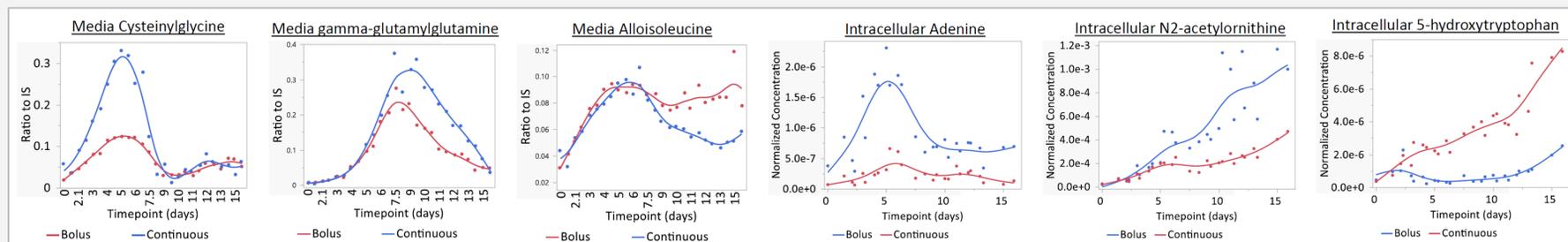
Product characterization of the expressed IgG was performed using the ZipChip Charge Variant Analysis Kit. Differences in charge variant profile and glycoforms were observed at the beginning and end of the culture. Glycation was measured and found to be lower in product from the bioreactor continuously fed with glucose.
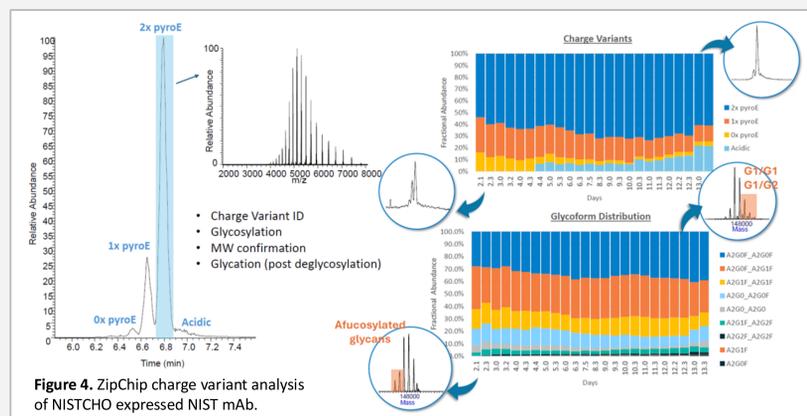

Figure 4. ZipChip charge variant analysis of NISTCHO expressed NIST mAb.

Figure 5. (left) Charge variant and glycoform distribution measured using the ZipChip charge variant assay. Measurements were made starting at day 2 through day 13.
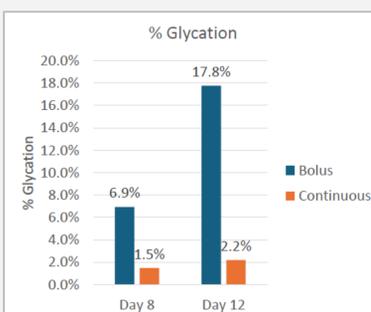

Figure 6. (left) %Glycation of the mAb product measured at day 8 and day 12 from each bioreactor.

Conclusions

- ZipChip System was used to quantify hundreds of media and intracellular metabolites from bioreactors
- Trends related to accumulation and depletion of metabolites were characterized in growth media and intracellular space
- Differential metabolism of analytes was observed when comparing bolus glucose feeding to continuous glucose feeding.
- ZipChip System can provide in depth metabolic characterization of CHO bioreactors to support process optimization, refinement, and fault detection.