Monitoring Amino Acid Composition of Cell Culture Media using Microfluidic CE-MS

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Overview

Monitoring amino acid content of cell growth media can provide important biological information but is challenging using established techniques. To achieve acceptable separation performance and detection these analytes are often derivatized to increase either their hydrophobicity, volatility, or UV absorbance properties for analytical techniques such as liquid and gas chromatography. The reliance on derivatization increases the time, complexity, and cost associated with the analysis making the assay impractical for routine use in many environments. By combining capillary electrophoresis (CE) separations with MS detection the derivatization step can be eliminated and the only sample preparation necessary is dilution. Here we present a simple and rapid assay for monitoring amino acids in growth media using microfluidic CE-MS.

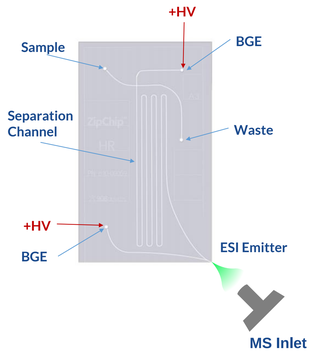
Methods

Sample Preparation. Bioreactor cultures of mammalian cells were grown using a ambr® 250 high throughput system (ambr Bioreactor System, Sartorius). Samples were derived from time points taken over the course of a 14 day growth cycle. The collected spent media time points were stored at -20 °C until analysis. Spent media samples were first diluted 1000-fold with LC/MS grade water to bring the concentration of media components into the linear dynamic range of the system. The diluted samples were then diluted an additional 10-fold with 80/20 water/methanol containing 100 mM ammonium acetate and 1 µM stable isotope labeled amino acids to serve as internal standards (Cambridge Isotope Laboratories, Inc.).

For quantitation, a calibration curve was prepared containing the 20 essential amino acids. The curve was created using 4 points at 0.5 µM, 5 µM, 50 µM, and 500 µM which were then diluted 10-fold with the solution containing internal standards, for a final concentration of 0.05 µM, 0.5 µM, 5 µM, and 50 µM.

Instrumentation. The ZipChip ™ Device was used for all analyses (908 Devices Inc.) ZipChips utilize microfluidic technology to harness the inherent speed and efficiency of zone electrophoresis separations. The microfluidic device design, as seen below, incorporates an injection cross, serpentine separation channel, and an integrated ESI emitter where electrospray is generated directly off the corner of the device. Uniform and stable surface coatings suppress the electroosmotic flow and yield highly efficient separations. Microfluidic chips with a 10 cm separation channel (HS, 908 Devices Inc.) and the Metabolite Assay Kit (908 Devices Inc.) were used for analysis. An on-chip injection volume of 5 nL was performed and the separation was run at a field strength of 1000 V/cm. MS analysis was performed using a Thermo Fisher Exactive Plus EMR Orbitrap mass spectrometer. Data was processed using Skyline (University of Washington).

Device Schematic



Bioreactor Growth Conditions

Three separate cell cultures were used to generate this data. Ammonia was added 12 hours post inoculation to two of the bioreactor vessels to stress the cultures during the growth cycle, while one vessel was left unperturbed to represent more standard growth conditions.

Amino Acid Quantitation

Microfluidic CE-MS is well suited for analyzing small polar molecules since the separation mechanism is based on charge and hydrodynamic radius rather than retention on a stationary phase. Figure 1 shows the separation of the t_0 timepoint, representing the initial starting concentrations of amino acids in the growth media. All amino acids are detected in less than 3 minutes.

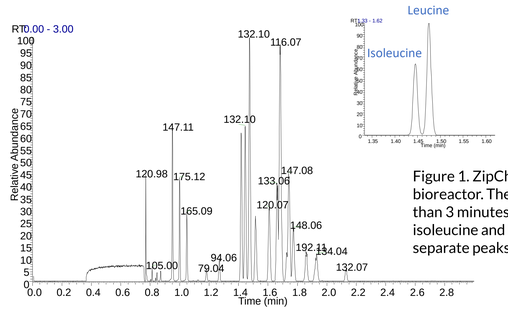


Figure 1. ZipChip separation of the t0 timepoint from the bioreactor. The amino acids migrate through the chip in less than 3 minutes. As shown in the inset, isomers such as isoleucine and leucine are resolved and detected as separate peaks for confident identification and quantitation.

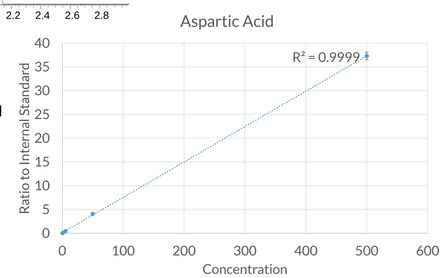


Figure 2. Representative calibration curve for amino acid quantitation. Calibration curves were generated by calculating the ratio of the amino acid peak to its stable isotope internal standard. Curve were generated from n≥3 measurements and all R^2 values were greater than 0.98.

Table 1. Characterization of the t0 timepoint from Bioreactor A

	Average [c] mM	%RSD
Lysine	2.3	4.7%
Arginine	1.2	1.3%
Histidine	1.0	3.6%
Glycine	1.3	6.3%
Alanine	0.1	23.8%
Valine	3.2	1.5%
Isoleucine	2.7	1.8%
Leucine	4.3	1.8%
Serine	5.1	3.1%
Threonine	2.6	1.9%
Methionine	1.3	1.6%
Proline	5.4	1.9%
Phenylalanine	1.3	1.5%
Glutamic Acid	2.0	5.3%
Tyrosine	0.8	1.8%
Aspartic Acid	1.5	1.8%
Asparagine	6.1	5.9%
Tryptophan	0.8	3.9%
Glutamine	4.0	6.7%

Table 2. Concentration of Valine at each time point from Bioreactor A

Time Post Inoculation (hrs)	Average [c] mM	%RSD
0	3.2	1.5%
36	3.8	1.8%
60	3.4	2.5%
84	4.5	2.9%
108	5.3	5.8%
132	6.0	4.5%
156	5.7	2.5%
180	6.6	3.5%
204	6.7	2.7%
228	6.6	3.6%
252	7.1	1.6%
276	7.6	2.5%
300	8.1	2.8%
324	8.8	3.8%
348	9.2	3.1%

Table 1 shows the starting concentration of amino acids in Bioreactor A. The amino acid content was quantified for each timepoint taken during the experiment. Representative data of an amino acid concentration over time is provided in Table 2. Timepoint data was collected for every amino acid in Table 1 for all three bioreactors. These measurements were used to generate plots of concentration over time.

Amino Acid Metabolism Over Time

The viable cell density was measured at 0 hrs, 12 hrs, and then approximately every 24 hours (Fig. 3). By this measurement, cell growth appears similar for Bioreactors A and B, but is decreased for Bioreactor C. Despite cell density appearing similar for A and B, the amino acid data shows that metabolism was different between the two cultures. Monitoring cell growth by tracking amino acid metabolism could signal bioreactor faults before an impact on cell viability is detected. Figures 4-7 show the change in concentration over time for select amino acids.

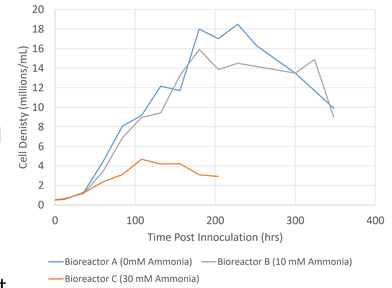


Figure 3. Viable cell density for bioreactors A, B, and C monitored over the course of cell growth. Time point measurements were taken approximately 12 hours apart.

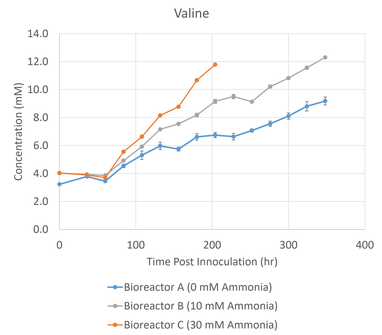


Figure 4: Concentration of valine over time. After ~60 hrs of growth the traces for the 3 bioreactors diverge. 10 other amino acids followed a similar pattern of gradually increasing in concentration over time.

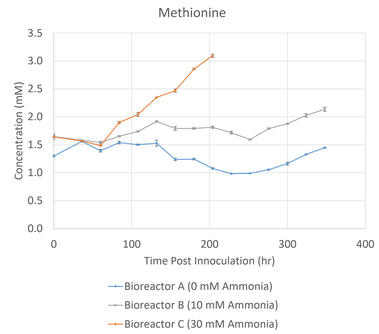


Figure 5: Concentration of methionine over time. For Bioreactor A, the concentration does not fluctuate as significantly during growth, but a greater amount of change is observed with Bioreactors B and C. Serine, Tryptophan, and Proline also follow this pattern.

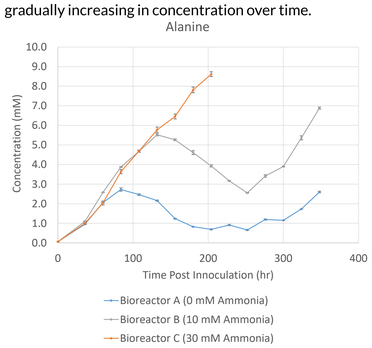


Figure 6: Concentration of alanine over time. For Bioreactors A and B, alanine initially increases, but then decreases in concentration as cell density increases. The concentration begins to increase again as the viable cell density decreases (see Figure 3).

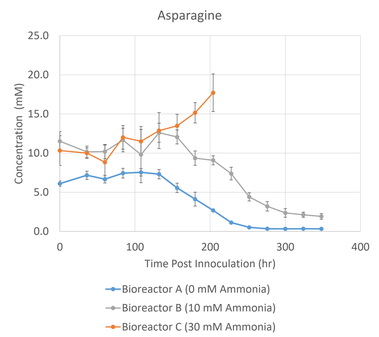


Figure 7: Concentration of Asparagine over time. As viable cell density increases, asparagine concentration decreases suggesting that it is nearly completely consumed by the cells during growth.

Conclusions

The ZipChip system can quickly and easily measure amino acids from growth media samples. The assay does not require a derivatization step or other complex sample prep techniques, and can be fully quantitative. By tracking their concentration over time differences in amino acid metabolism were observed from three different bioreactor conditions. This information could be used to better characterize optimal growth conditions or detect faults earlier in the production process.