Rapid at-line nutrient profiling from an ammonia stressed CHO cell line utilizing an integrated benchtop analyzer

Ji Young L. Anderson,[1] Kathryn Elliott,[2] Cameron Schnabel,[2] Colin Gavin,[1] Kenion H. Blakeman,[1] Sarah W. Harcum,[2] Glenn A. Harris[1]

1) 908 Devices Inc., 645 Summer St., Boston, MA 02210 2) Clemson University, Dept. of Bioengineering, 208 Biosystems Research Complex, Clemson, SC 29634

Despite intense efforts in improving Chinese hamster ovary (CHO) productivity via media optimization, concerns regarding feeding strategies, cloning techniques, low product titers, and CQAs are still prevalent. Scale-up is confounded by incompatible process analytical technologies (PAT) that are used between small-scale bioreactors (i.e., ambr®250) and manufacturing-scale bioreactors. The new analyzer shown here requires minimal sample volume (10 µL) and no sample derivatization making it well suited to users of microbioreactor platforms.

Methods and workflow

samples

Analytics used in the study

Beckman ViCell for cell density and viability

Roche Cedex Bio for glucose, lactate, NH_3, IgG

The Rebel for amino acids, vitamins, dipeptides, amines

A 14-day batch fed process of the CHO NIH VRC01 cell line was run on the ambr®250 platform. Two bioreactors per each ammonia (NH_3) stress level (0 mM, 10 mM, 30 mM) were grown. Samples were automatically retrieved with the bioreactor robotics for at-line analysis with the platforms shown here. Of note, was the sample prep for the Rebel for spent media analysis - all samples were centrifuged to remove cells, diluted 100X with diluent premixed with internal standards, and loaded into the analyzer.

Culture analytics and discussion

Viable cell densities (VCD), cell viability, lactate and glucose values between the 0 mM and 10 mM NH_3 stressed bioreactors were very similar with the only difference in NH_3 levels explained by the addition of the small of NH_3 to bioreactors 3 and 4 after t = 12 hrs. There were more pronounced changes in the values for the 30 mM NH_3 stressed bioreactors than the 0 mM and 10 mM bioreactors. IgG concentrations were the most defining general characteristic of culture changes between all three culture conditions run.

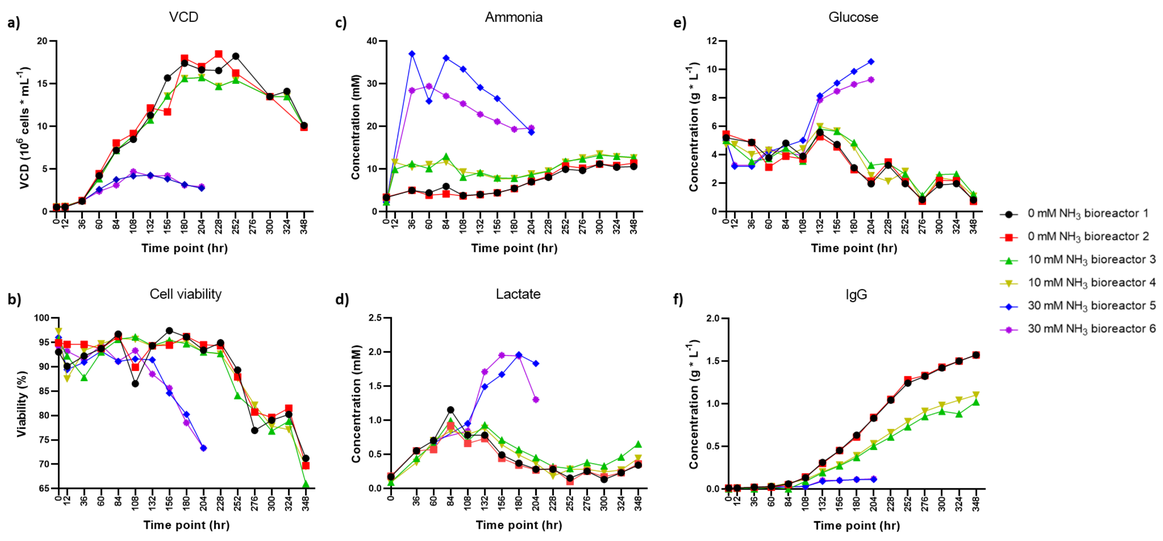

Figure 1: Traditional process analytics showing the viable cell density (a), cell viability (b), ammonia (c), lactate (d), glucose (e) and IgG (f) levels of the six cultures.

With the spent media analysis data, there was a net increase in the amino acid levels of Ala, Arg, Asp, Glu, Gly, His, Ile, Leu, Lys, Phe, Thr, Trp, Tyr and Val in all bioreactors with Gly levels showing increases in excess of 8X initial levels in all bioreactors. In contrast, other media components either steadily decreased in concentration or were completely depleted by the end of culture. For example, Asn was depleted in all 0 mM and 10 mM bioreactors but was at ~2X the initial levels in the 30 mM stressed bioreactors at the end of culture. Also, the 30 mM condition may have caused either complete degradation or rapid consumption of choline in the 30 mM bioreactors since it was no longer present by the t = 36 hr sampling.

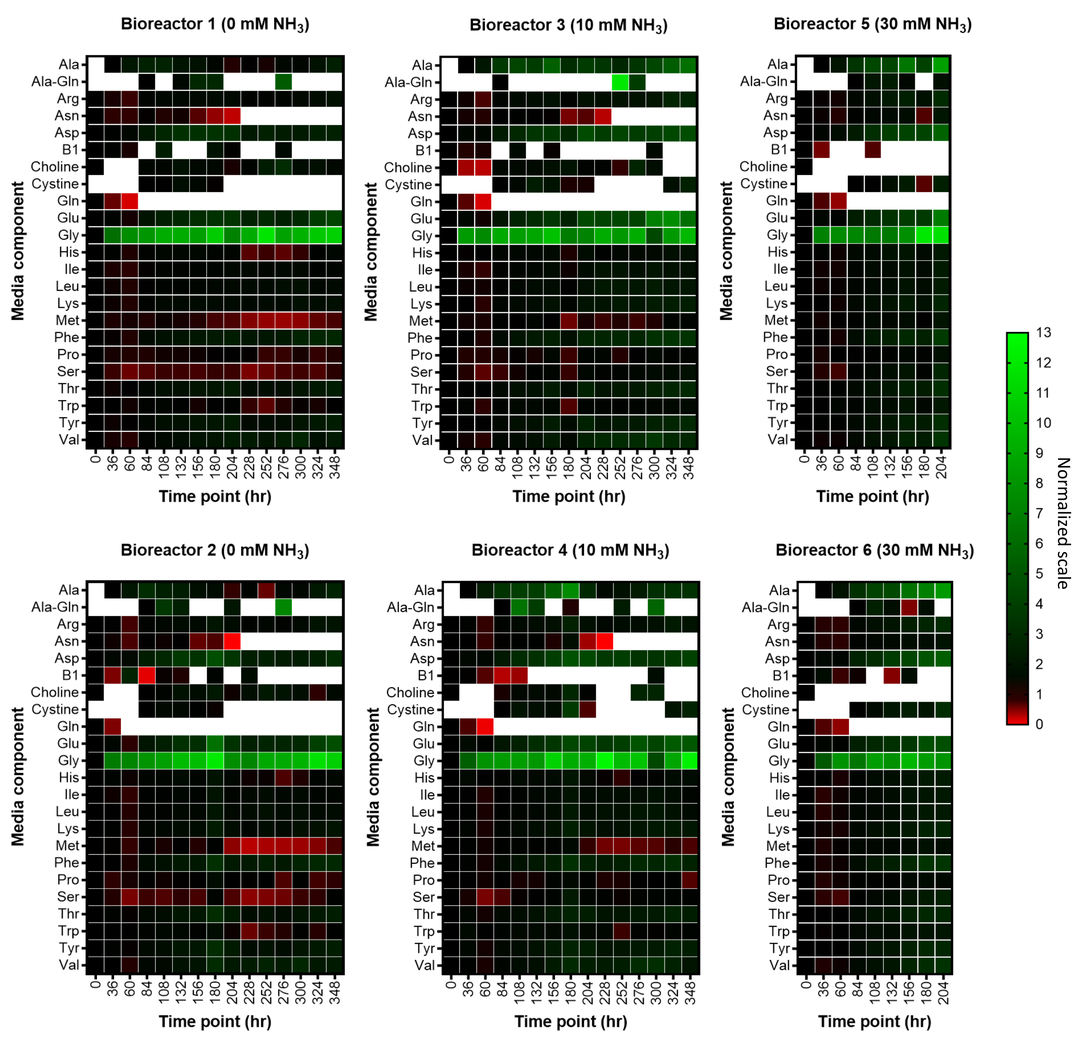

Figure 2: Spent media analysis of the six cultures. White boxes represent when the component was not detected. Scale is normalized to t = 0 hr for all components except Ala, Ala-Gln and cystine when t = 36 hr was used).

Clemson Univ. would like to acknowledge the support from the National Science Foundation (NSF) under Grant No. OIA-1736123. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the NSF. Both 908 Devices Inc. and Clemson Univ. would also like to thank the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) for creating environments where groups like ours can meet and collaborate.

SCAN ME

908devices