Evaluating the Impact of Glucose Feed and Temperature Modulation of Bioreactors Using Microfluidic CE-MS for Metabolic Profiling and Product Characterization

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Introduction

Bioprocess optimization is facilitated by in-depth knowledge of the bioreactor conditions as well as the expressed product. In this work we present analysis of the monoclonal antibody (mAb) product and metabolic conditions present in CHO fed-batch bioreactors cultured under different glucose feed and temperature conditions. Microfluidic CE-MS based methods were used to collect data from spent media samples taken from five benchtop bioreactors; methods deployed focus on simplicity and ease-of-use while maintaining high data quality. Product quality attributes were measured from the expressed mAb with only simple dilution. Media metabolites were quantified using microfluidic CE-MS, with trends analyzed against at-line bioreactor measurements to probe differences in metabolism between the culture conditions used.

Methods

Bioreactor Setup:

NISTCHO cell lines expressing NISTmAb were cultured in benchtop bioreactors over 14 days. The bioreactors were continuously fed glucose at 1 g/L, 3 g/L, or 5 g/L with a temperature of 30° C, 33.5° C or 37° C at the stationary phase of growth. Samples of media were removed ~2x daily and centrifuged to remove cells. Glucose and lactate were measured at-line using a Nova Flex 2 (Nova Biomedical). Viable Cell Density was measured using a Vi-CELL XR (Beckman Coulter).

Metabolite Extraction and Analysis:

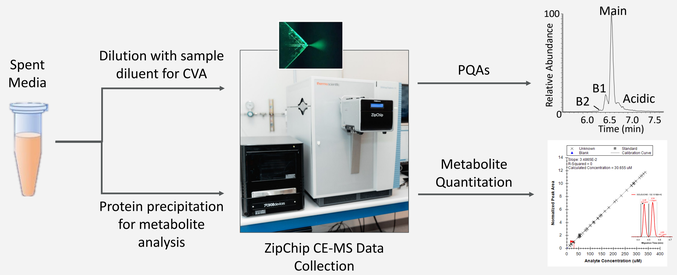
Spent media was diluted 10x with PBS. Metabolites were extracted using the following method:

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

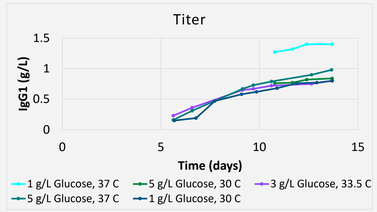
Quantitation was performed using single point calibration with a matrix-based calibration material. Data collection performed using a ZipChip Interface (Repligen Corp.) on an Exploris 240 Biopharma mass spectrometer (Thermo Fisher Scientific). The system was primed with Peptides BGE (Repligen Corp.) and an HR chip (Repligen Corp.) was used for analysis. Raw files were processed using a Skyline v24.1 (University of Washington) template containing target molecules with an in-house built algorithm for peak assignment correction. Statistical analyses and trend plots were generated using JMP 17.0.0 (SAS Institute) or Excel (Microsoft).

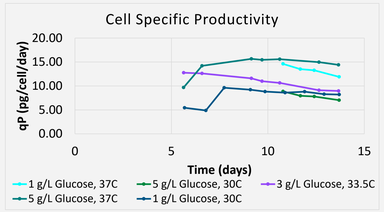
Charge Variant Analysis:

Charge variant analysis was performed using the Charge Variant Analysis Kit (Repligen Corp.), and HRN chip (Repligen Corp.), and a ZipChip interface coupled to an Exploris 240 Biopharma MS (Thermo Fisher Scientific). Spent media samples were diluted with sample diluent from the CVA kit. For glycation analysis mAbs were deglycosylated using Glycinator (Genovis Inc.) enzyme added directly to the media and incubated at 37 C for ~1 hour followed by dilution with CVA Kit sample diluent. Data was processed using Biopharma Finder 5.0 (Thermo Fisher Scientific) and UniDec deconvolution software. Charge variants were identified via mass and mobility shifts between CE peaks. Glycoforms were identified via accurate mass and mass shifts within CE peaks.



Bioreactor Performance





Analysis of titer revealed one condition produced significantly more mAb product than the other conditions. However, when cell specific productivity (qP) was calculated the performance of the different cultures was more comparable. Following this, product quality attributes (PQAs) were analyzed to determine what impact the different conditions had on the product outside of just titer.

PQA Monitoring with CVA-MS

Representative ZipChip CVA-MS from day 9 of a bioreactor:

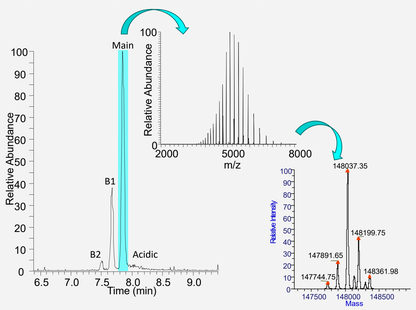


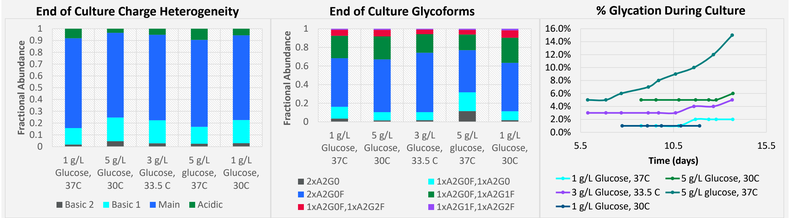
Table 1. Identified glycoforms of the expressed mAb

Glycoform	Average Mass (Da)	Theoretical Mass (Da)	Matched Mass Error (ppm)
G0F_G0F	148037.34	148037.16	1.2
G0F_G1F	148199.77	148199.30	3.1
G0_G0F	147891.66	147891.02	4.3
G1F_G1F	148361.98	148361.44	3.7
G0_G0	147744.75	147744.88	0.9

Table 2. Identified charge variant PTMS of the expressed mAb

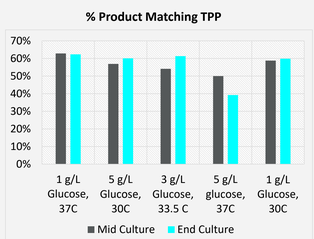
Variant	PTM Identification	
B2	0x pyroE	pyroglutamic acid
B1	1x pyroE	pyroglutamic acid
Main	2x pyroE	pyroglutamic acid
Acidic	deamidation	

Mass spectra for each charge variant peak in the ZipChip separation were deconvoluted to obtain masses. Mass shifts within the deconvoluted spectra of the main variant were used to identify glycoforms (Table 1). Mass shifts between the charge variants and shifts in migration times are used to identify the PTMs causing the charge heterogeneity (Table 2).



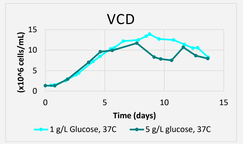
Assessing Product Quality

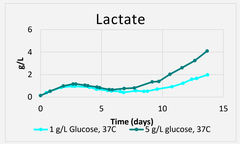
A target product profile (TPP) was developed that favorably weighted main variant abundance and galactosylated glycan abundance and unfavorably weighted glycation. After applying this to the resulting product from the 5 bioreactors the culture continuously fed 1 g/L glucose at 37° C yielded the highest amount of mAb matching the TPP while the 5 g/L glucose 37° C conditions yielded the lowest despite having the highest qP. This is primarily due to the significantly higher levels of glycation observed for that bioreactor.

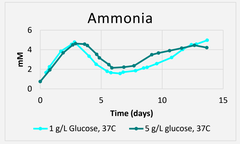


Metabolic Profiling

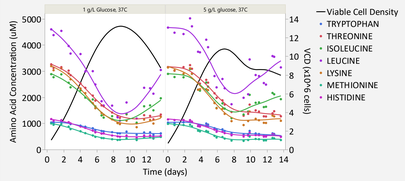
The bioreactors having the highest and lowest amount of mAb product matching the TPP were evaluated to determine if there are significant differences in their metabolic profiles. Viable cell density (VCD), lactate and ammonia were measured at-line during cell culture while media metabolites were quantified from time points after the cultures were complete.



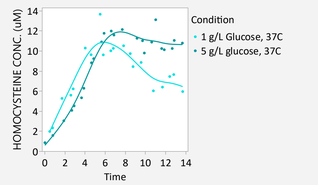


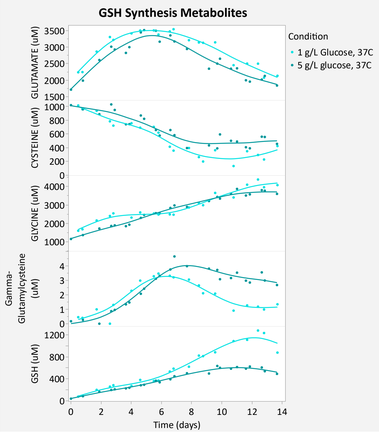


Many commonly measured metabolites differ slightly in their metabolism but follow a similar general trend.

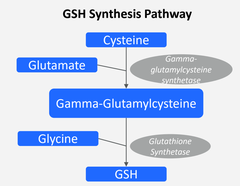


Homocysteine metabolism differs and its accumulation has been shown to inhibit cell growth.[1,2]





Differences in some of the key metabolites in the Glutathione (GSH) synthesis pathway were observed. The 1 g/L glucose, 37 C bioreactor accumulated higher levels of GSH by the end of culture. Upregulated GSH pathways have been linked to higher mAb production and better performing cell cultures.[3,4]



Summary

- 5 different culture conditions were used to express NISTmAb from the NISTCHO cell line. After assessing the bioreactor cultures based on titer and qP, product quality attributes and media metabolites were evaluated.
- ZipChip CVA analysis of the expressed NISTmAb identified 7 charge variants and multiple glycoforms from the mAb product.
- Comparison of the PQAs against a TPP determined that the culture continuously fed glucose at 1 g/L, 37 C resulted in the highest levels of mAb matching the TPP.
- Metabolic analysis of the cultures revealed observable differences in metabolism with homocysteine and GSH synthesis metabolites highlighted here.

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References:

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2. Mulukutla, B., et. al. Identification and control of novel growth inhibitors in fed-batch cultures of Chinese hamster ovary cells. *Biotechnol Bioeng*. **2017**, 117, 1179-1790.
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4. Chevallier, V. et. al., Characterization of glutathione proteome in CHO cells and its relationship with productivity and cholesterol synthesis. *Biotechnol Bioeng*. **2020**,117, 3448–3458.