Use of Metabolic Flux Analysis to Expedite Media Selection and Optimization for CHO Clones

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Introduction

Within the biomanufacturing industry, screening and optimizing media is a lengthy and labor-intensive process. While media components impact cellular metabolism, it is not always understood what metabolic changes are occurring at an intracellular level. Metabolic Flux Analysis (MFA) with ^{13}C glucose was used to understand how media, cell lines, and culture methods impact the cellular metabolism. Utilizing metabolic flux rates to classify CHO clones can lead to a better understanding of cellular metabolism and may accelerate media optimization. We hypothesized that CHO clones could be 'clustered' based on metabolic profiles derived from MFA leading to a more informed starting point for media optimization, resulting in a shorter timeline to a clone-specific optimized medium when compared with more traditional design of experiment approaches (Figure 1).

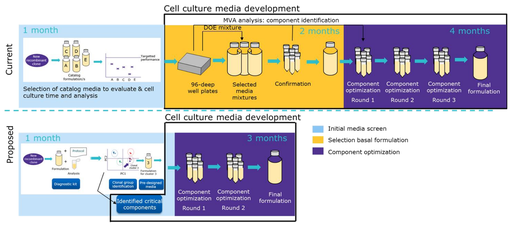

Figure 1: Media optimization timeline reduction with MFA

Methods

MVA Clonal Grouping
Twenty-three diverse CHO clones were tested in high throughput library feed screens to evaluate VCD, specific productivity (q_p), and titer responses and then analyzed using a Multivariate Analysis (MVA) approach to predict clonal groups. Optimal feeds were predicted for each group using Design-Expert® and all predicted feeds were tested on a subset of the 23 clones to determine if the best feed for each clone matched the one predicted for the clonal group.

MFA Testing
For MFA testing, 12 cell lines selected from the four different clusters found by MVA (Table 1) were tested following the process as outlined in Figure 2. A supporting study was conducted to demonstrate that cellular metabolism was comparable when cultured in a 50 mL tube (TPP ® TubeSpin® Bioreactor) and in a 1L bioreactor (data not shown).

Cells were cultured with U-^{13}C glucose (Cambridge Isotope Laboratories) for 72 hours during both growth (Day 1 to 4) and production (Day 7 to 10) phases of a fed batch assay and analyzed to determine the point at which ^{13}C consumption stabilized within the sample, indicating they had achieved steady state. Before ^{13}C inoculation, non-labelled glucose additions were made as needed to support cells. A time zero (T0-) sample was taken prior to ^{13}C introduction to the culture, followed by measuring glucose concentration so that the ^{13}C glucose quantity added would achieve a 50:50 concentration of ^{13}C glucose to standard glucose. 30 mins after the introduction of ^{13}C, an additional sample (T0+) was taken. For the next three days, the CHO cells were harvested at intervals of 48, 64, 68, and 72 hours during each phase.

At each harvest point, a sample was taken from the culture and cell pellets were separated from the media. Spent media was quantitively analyzed for glucose, lactate, and amino acids. Cells were quenched and extracted in ice-cold methanol and cell extracts analyzed by LC/MS to determine the amount of ^{13}C incorporated into 30 to 50 metabolites found within various metabolic pathways of interest and representing several classes of metabolites. VCD and titer were also used for calculating flux estimates.

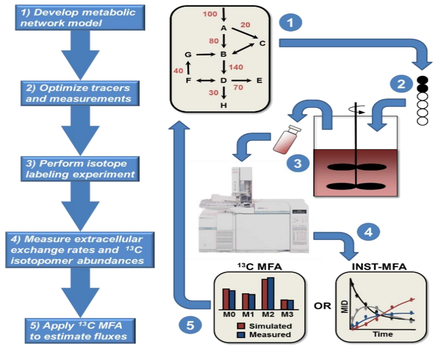

Figure 2: MFA process for calculating flux rates

MFA Model
MFA analysis was performed by Metalytics, Inc, using CoreMFA™, a software-enabled technology for metabolic flux analysis for metabolic profiling. A metabolic model as illustrated in Figure 3 was constructed for use in this project. The model represents central carbon metabolism through glycolysis, pentose phosphate pathway (OPPP), and the tricarboxylic acid (TCA) cycle along with the metabolism of fatty acids and amino acids. The model also includes the uptake and/or excretion of amino acids, lactate, and glucose along with algorithms to represent biomass and the primary protein or monoclonal antibody product. Using this model, along with the data described above, metabolic flux rates were calculated with CoreMFA using a stationary MFA methodology at 48 and 72 hours within each phase.

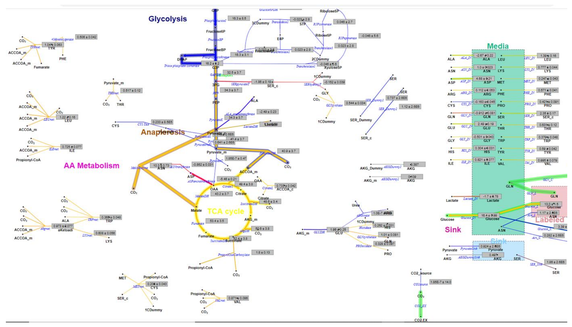

Figure 3: MFA model and comparison of two cell lines

MFA Model Validation

Using the calculated flux rates from MFA for each clone at each time point in both phases, the clones were separated into groups using 2 different methodologies, MVA and hierarchical clustering. Design Expert® was then used to predict an optimized feed for each group with both methodologies. For validation, each clone was grown in fed batch culture with each of the predicted feeds to obtain VCD and titer data which was used to select the best clustering method.

Results

These results clearly demonstrate that CHO clones with the same genetic background and producing the same product have distinct metabolic profiles. MVA was used to predict four clonal groups for the 23 clones based on the results of feed library screening. Cell line, protein produced, and transfection of each clone did not correlate to individual groups (Table 1). Clonal groups were selected by specific productivity (q_p) and a predicted optimal feed was found for each group (Figure 4A). 20 cell lines were tested with all predicted feeds, but feeds B and D were similar in composition and the most preferred across all clones (Figure 4B). MVA generated groups had limited success at feed prediction for the clonal groups.

Clone	Molecule	Background	Titer	VCD	qP
DuxB11	H	DuxB11	A	A	A
EPO c33	C	CHOZN	D	D	A
15-4	A	CHOZN	B	B	B
23B6	A	CHOZN	B	B	B
2F4	A	CHO-K1	D	C	B
18-9	A	CHOZN	C	C	B
18-17	A	CHOZN	C	C	B
41-2	B	CHOZN	C	C	B
18-15	A	CHOZN	B	C	B
13G10	A	CHOZN	D	D	B
c23	B	CHOZN	D	D	B
EPO c10	C	CHOZN	C	D	B
13F1	A	CHOZN	D	C	C
13E4	A	CHOZN	B	C	C
2F5	A	CHO-K1	D	D	C
13E7	A	CHOZN	C	A	D
3A6	D	CHOZN	D	C	D
118-28	E	CHOZN	C	C	D
92-6	E	CHOZN	C	C	D
SP5	B	CHOZN	C	C	D
15-5	A	CHOZN	D	D	D
CHO-S	F	CHO-S	D	D	D
DG44	G	DG44	D	D	D

Table 1: Clonal grouping by MVA by multiple outputs

Figure 4: Predicted feeds for clonal groups (A) and validation with predicted feeds (B)

Metabolic differences were clearly identified by comparing calculated flux rates as shown with color coding between 2 clones in Figure 3. Clones were clustered using the MFA calculated flux rates using two grouping methodologies. Each method created 4-5 clonal groups and an optimized feed was identified for each group. With the overlap in optimal feeds between groups, all 5 predicted feeds were tested with 11 cell lines to identify which was best for each cell line. The MVA grouping method, which had two optimal feeds for the 4 groups, resulted in better predictions than the hierarchical clustering. The clonal grouping with the flux rates for the T48 time point matched the predicted results closely while at the T72 time point three clones fell outside of the predicted groupings (Figure 5A-B and Table 2). T48 shows the strongest grouping prediction, and both timepoints are more consistent than previous attempts using only MVA analysis.

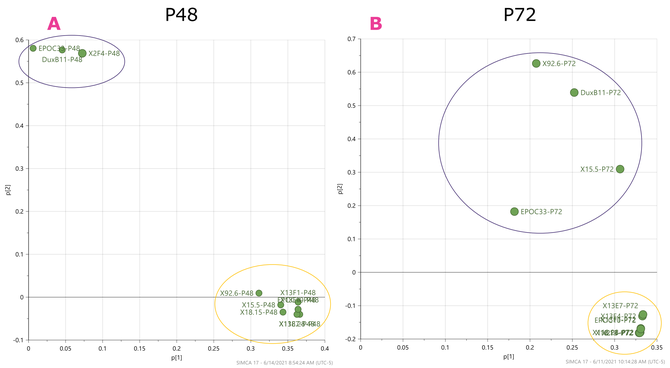

Figure 5: Predicted clonal groups by MFA for 48h (A) and 72h (B) in the production phase

Clone	P48 group	P72 group	Best feed
DuxB11	Feed 2	Feed 2	Feed 2
EPO c33	Feed 2	Feed 2	Feed 2
2F4	Feed 2	Feed 1	Feed 2
18-15	Feed 1	Feed 1	Feed 1
EPO c10	Feed 1	Feed 1	Feed 1
13F1	Feed 1	Feed 1	Feed 1
13E4	Feed 1	Feed 1	Feed 1
13E7	Feed 1	Feed 1	Feed 1
118-28	Feed 1	Feed 1	Feed 1
92-6	Feed 1	Feed 2	Feed 1
15-5	Feed 1	Feed 2	Feed 1

Table 2: Clonal grouping predicted by MFA compared to validation results

Conclusion

This work demonstrates the usefulness of MFA for identifying optimal media for cells. Cellular metabolism was similar in both bioreactors and bioreactor tubes for fed batch, indicating high throughput analysis is possible for MFA testing, and confirming the ability to utilize small-scale models, saving resources and reducing costs for screening media and modeling metabolic fluxes. Crucially, the MFA models were able to sort the tested clones into distinct groups, each with an associated feed that is better for most cells in the group for protein production. Testing with additional clones is underway to confirm other clones can be properly sorted into the clonal groups for accelerated media optimization. Success in using these MFA models for clonal grouping and other diagnostic media identification strategies can greatly reduce the timeline and resources needed to optimize media for CHO cells.

Acknowledgments
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