Characterizing Basal and Feed Media Effects on Mammalian Cell Cultures by Systems Engineering Approaches

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Abstract: Choosing the right cell culture media to improve the culture performance is one of the major challenges in *Biologics process development*. In this regard, we investigated the effect of basal and feed media on cell growth, productivity, and cellular metabolism during CHO-K1 cell culture, which are the main workhorse in the biopharmaceutical industry. We elucidated how the cell performance changes under different combinations of basal and feed media (2 x 2) conditions. To do so, we exploited systems engineering approaches that four hierarchical steps which are data collections, multivariate statistical analysis, *in-silico* flux analysis with genome-scale metabolic model, and knowledge-based targeting media components. Our findings indicate that feed media have a much greater impact on cell growth and production than basal media. The framework characterized the major metabolic bottlenecks in central carbon metabolism and identified new manipulatable target components of the media reformulation in the feed that impeding improved growth.

Keywords: Biopharmaceutical processes, Media formulation, CHO cell culture, Systems approach

1. INTRODUCTION

Industrial Chinese Hamster Ovary (CHO) cells are mainly cultured in fed-batch mode with well-designed media formulations, which influences such parameters as cell growth, protein production, and post-translational modifications (Gronemeyer et al., 2014). For many years a recognized critical bottleneck in development of recombinant protein production is the reformulation and/or optimization of medium to increase cell density and protein titer (Combe & Sokolenko, 2021). Especially, choosing the right combination of media and feed supplements is a key toward achieving final therapeutic product in good yield and bioprocess efficiencies during CHO cell culture (Pan et al., 2017). Since cells become self-limiting, media redesign and optimization benefits fine-tuning of certain media components to eliminate cellular bottleneck impacting growth and production (Park et al., 2018). As the needs of the understanding of cell metabolism have grown so has culture media development and formulation technology. Such disciplines as genome-scale metabolic flux analysis and statistical analysis are now enabling efficient fine-tuning of nutritional requirements, harmonization of media and feeds, and feed strategies. In addition to their contributions toward rising titers, media and feeds have significantly and positively influenced productivity attributes for therapeutic proteins (Torkashvand et al., 2015). Moreover, recently, manufacturing of biopharmaceuticals requires significantly more rigorous characterization than conventional therapeutic monoclonal antibodies (mAbs) through consistent and reproducible bioprocess to meet productivity attributes (Webster et al., 2021). It is required to screen multiple media constituents and identify promising candidates followed by tailoring the bioproduction media and supplements to achieve optimal cell performance within a short timeline. Also, carefully designed sets of media or combination of media and feed supplements provide the best environment for cell culture because clonal cell lines may have pathway-specific differences toward achieving high viable cell densities and productivity. To meet the best environment for cell culture, each CHO cell culture medium typically contains 70-100 components, which are then tested using high-throughput experimental approaches with application of design of experiment methodology (Rouiller et al., 2013). This process generates a massive data for multiple components, which is then used for reformulation and/or optimization. This is, however, very labor-intensive, costly, and time-consuming.

In this regard, to investigate the effect of basal and/or feed media on cell growth, IgG productivity, and cellular metabolism during CHO cell culture through a systematic framework. we developed the systematic media redesign framework consists of four hierarchical steps which as culture data collections, multivariate statistical analysis, *in-silico* flux analysis with genome-scale metabolic model, and knowledge-based targeting media components. In this way, we could reveal new manipulatable target components of the media reformulation or highlight the cellular/process bottleneck that leads to better performance without exhaustive reliance experimental data, taking into account manufacturing cost cutting by reducing unnecessary laboratory testing. In addition, this systematic framework in *Biologics process development* could be a successful computation-driven model approach for rising cell growth and productivity with consistent product quality.

2. EXPERIMENTAL METHODOLOGY

2.1 CHO Cell line, Media, and Cell Culture Conditions

For seed trains, a proprietary recombinant Immunoglobulin G1 (IgG1) antibody producing CHO-K1 cell was first thawed from a frozen vial and inoculated in a proprietary chemically defined CELLiST™ basal medium 'Medium C' (Ajinomoto Genexine, Co., Ltd.) for two passages, followed by three passages in three different basal media ('Medium A', 'Medium B' and 'Medium C'), prior to start of main production run. All media was designed and manufactured by Ajinomoto Genexine Co., Ltd. It is important to note that media designations 'A', 'B' and 'C' are arbitrary and do not represent specific commercial CELLiST products. Culture was supplemented with 2 mM of L-glutamine (ThermoFisher Sci.), 30 ug/mL of puromycin (ThermoFisher Sci.), and 5 mg/L of insulin (Sigma-Aldrich). Cells were seeded at viable cell density of 3 x 10^5 cells/mL into 30 mL media in 125 mL Erlenmeyer shake flasks (Corning Life Sciences). Flasks were placed on an orbital shaker (115 rpm with 22 mm width) in humidified incubator at 5% CO_2 in air at 37°C. Before production run, three basal media (A, B, C) and three feed media (1, 2, 3) as different combinations were screened. Four combinations (basal A and B with feed 1 and 2) with high VCA were selected to investigate the effects of different basal media and feeds on cell growth and specific productivity during the entire production run. For production run in these four different media conditions, fed-batch culture was performed using the 15 mL automated mini bioreactor system (Ambr15®, Sartorius). The ambr reactors were cultured for 14 days with 10 mL initial working volume. Physical setting parameters were controlled via ambr Runtime software as 37°C, stirrer speed of 1200 rpm, DO 50% and pH 7.0 ± 0.05. Cells were inoculated at 3 x 10^5 cells/mL in 10 mL medium for each vessel. The pH was controlled by sparged CO_2 and addition of 2 N NaOH. When glucose was below the 4 g/L, glucose was topped up to 6 g/L. Culture was supplemented once every two days with two different proprietary feed media 'Feed 1' and 'Feed 2' depending on each condition from culture day 4 at 4% (v/v) ratio. These designations '1' and '2' are arbitrary and are not related to actual brand names.

2.2 Metabolite Profiles

Cell culture samples were collected daily for cell count and viability determination, and every two days for metabolite and amino acid concentrations. Viable cell density (VCD) and viability were measured with Cedex HiRes analyzer (Roche CustomBiotech). Glucose, lactate, ammonia, and IgG concentration were measured with Cedex Bio analyzer (Roche CustomBiotech). Amino acid concentrations were analyzed by ARACUS advanced analyzer (MembraPure), following the manufacturer's protocol for cell culture samples.

2.3 Data Evaluation

Data preprocessing and elemental balancing were carried out with the residual concentration of amino acids and differential integral of viable cell density. The residual concentrations were converted into the cell specific consumption and secretion rates (unit of "mmol/gDCW-hr" or "pmol/cell-day") to proceed preprocessing. VCD and IgG titer were calculated specific growth rate (SGR) and specific productivity (Q_P), respectively, and then incorporated into elemental balancing procedure as described in our previous study (Hong et al., 2018). From public database, in-house data and earlier studies (Bonarius et al., 1997; Hefzi et al., 2016; Sheikh et al., 2005), obtained biomass composition and IgG composition were utilized in the elemental balance calculation in order to calculate the carbon-specific input and output.

2.4 Multivariate Data Analysis (MVDA)

As for processed data, principal component analysis (PCA), partial least square (PLS) and PLS discriminant analysis (PLS-DA) were performed by SIMCA® software (version 16, Sartorius-Stedim, Umea, Sweden). The processed dataset includes specific growth rate (SGR), specific productivity (Q_P), specific consumption/production rate of amino acids, glucose, ammonia, and lactate. This dataset was assorted in culture day points (day 0, 2, 4, 6, 8, and 10) and phases (days 2-4, days 4-6, days 6-8, and days 8-10). In order to investigate the effect of basal and/or feed media, "*before feeding*" phase (days 2-4) and "*after feeding*" phase (days 6-8) were selected for further analysis. It should be noted that days 4-6 phase was excluded for the analysis since this phase is observed as transitional phase after feeding on day 4 based on the culture profiles. The data matrix in PLS model composed specific rates of metabolites as input (X) variables, and SGR and/or Q_P as output (Y) variables. The influence of an X variables on an output variable and the relative positive and negative correlation among input and output variables were quantified via variables in projection (VIP) scores and coefficient correlation, respectively. The input variables which have a VIP score greater than 1.0 were considered as a statistically significant among four culture conditions and the top 5 metabolites were selected as highly correlated to output variables.

2.5 Flux Balance Analysis

A latest genome-scale metabolic model (GEM) of CHO cells (*i*CHO2291) was utilized for the flux balance analysis (FBA). It should be highlighted that incorporating both enzyme kinetic parameters (e.g., k_{cat}) and experimentally measured specific

rates were constrained the enzyme-capacity constrained FBA (ecFBA) (Yeo et al., 2020). The ecFBA was implemented in COBRA Toolbox version 3.0 (Heirendt et al., 2019) for MATLAB R2020a. The Gurobi 9.1.1 solver was used for linear programming problems (http://www.gurobi.com). For obtaining condition-specific flux distribution, GEM is constrained with specific rates of amino acids, glucose, lactate, ammonia, and productivity. Biomass equation is maximized as an objective function for overall simulations. To consider experimental perturbation and avoid mathematical infeasible solutions, 10% relaxed range of consuming rates of nutrients as upper and lower bounds. For productivity, fixed rate of productivity is used as constraint. Within 10% of constraint range, we sampled 5,000 solutions on each condition and calculated the mean and standard deviation values. In order to decide which networks should be analyzed, we presented flux ratio on the visualized network map using the CHO GEM and rendered into the entire CHO network graphs using Cytoscape (version 3.9.1, https://cytoscape.org) (Shannon et al., 2003). At first, for comparison of *before* and *after feeding* with flux ration heatmap, we calculated relative flux ration as following equations (1) and (2). Secondly, we conducted narrow down procedures with thousands of metabolic network reactions. Here, note that we filtered minor fluxes which have negligible metabolic flux under 0.01. According to equations (3), (4) and (5), 51 noticeable metabolic reactions were selected for further analysis, which are not exchange or transport reactions among 4,334 global reactions with non-zero fluxes.

$$\frac{v_{i,A1} + v_{i,A2}}{v_{i,B1} + v_{i,B2}} (before\ feeding\ flux\ ratio) \quad (1)$$

$$\frac{v_{i,A1} + v_{i,B1}}{v_{i,A2} + v_{i,B2}} (after\ feeding\ flux\ ratio) \quad (2)$$

$$\left(v_{i,\min(all)}\right)\ or\ \left(v_{i,A1} + v_{i,B1} + v_{i,A2} + v_{i,B2}\right) > 0.01 \quad (3)$$

$$\frac{v_{i,A1} + v_{i,B1}}{v_{i,A2} + v_{i,B2}}, \frac{v_{i,A1}}{v_{i,A2}}, \frac{v_{i,B1}}{v_{i,B2}} > 1.2 \quad (4)$$

$$\frac{v_{i,A1} + v_{i,B1}}{v_{i,A2} + v_{i,B2}}, \frac{v_{i,A1}}{v_{i,A2}}, \frac{v_{i,B1}}{v_{i,B2}} < 0.8 \quad (5)$$

3. A SYSTEMATIC STRATEGY OF CELL CULTURE MEDIA AND FEED DEVELOPMENT

We transformed trial-and-error practice into systematic framework for advanced media formulation. We focused on establishing a sustainable framework for data-driven model-guided media development to design robust processes and consistent products, and to resolve a recurrent metabolic bottleneck (Figure 1). We exploited systems engineering approaches that four hierarchical steps which are data collections, multivariate statistical analysis, *in-silico* flux analysis with genome-scale metabolic model, and knowledge-based targeting media components. Firstly, the raw data of cell culture such as VCD, viability, titer, and residual media concentrations of nutrients are collected and processed by daily off-line measurements. Culture performance (i.e., specific growth rate and productivity) is evaluated and therefore allows a hypothesis on contributions of key nutrients. Then the processed data are incorporated with multivariate data analysis (MVDA) to find out noticeable component(s) making a close correlation with the culture performance. Subsequently the information of indicated components facilitates examination of intracellular flux effects on culture performance in combination with model-guided analysis, such as ecFBA. Knowledge on key components and metabolic bottlenecks enables us to suggest adjusting target metabolic contents in culture media and/or feed supplements could be made, therefore providing an adequate insight for advanced culture performance.

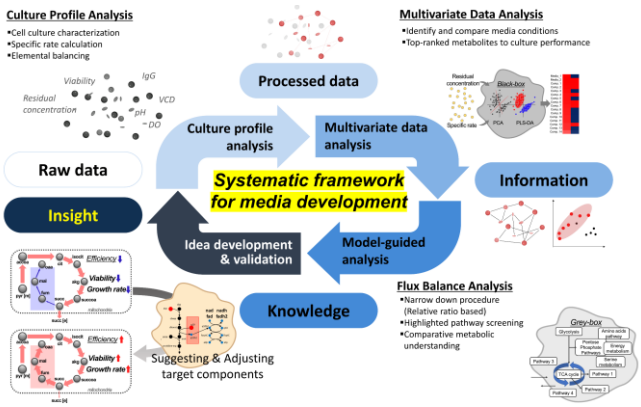

Figure 1. Scheme of systematic framework for data-driven model-guided media development.

4. RESULTS AND DISCUSSION

4.1 CHO Culture Performance in Different Media Conditions

Cell culture has shown different profiles depending on the varying basal and feed media conditions (Figure 2).

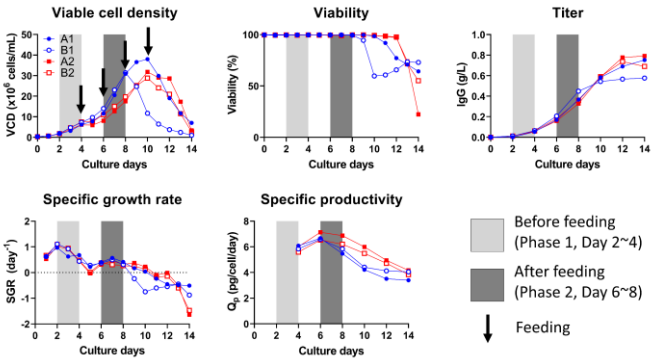

Figure 2. Effect of media and feed types on viable cell density, viability, titer, specific growth rate, and specific productivity over 14 days cultures. Solid and empty marks indicate basal A and B media conditions, respectively. Blue circles indicate Feed 1 and red boxes indicate Feed 2. Arrows indicate the feeding. Grey and dark grey boxes highlight the *before* and *after feeding* phases.

Before feed was supplemented to the culture (phase1; day 2-4) the profile seems to be similar in all conditions, however after the feed was added (phase2; day 6-8), noticeable deviation emerge both in cell growth and productivity profile. In cultures

with Feed1, viable cell density rapidly rises in exponential phase, while its rise lagged in cultures with Feed2. Specific productivities were high in Feed2 cultures and were slightly lower in Feed1 conditions. A trade-off like behavior was observed, for instance, the Feed2 condition seems to be related with lagged growth, otherwise enhancing productivity. Focusing on these diverging profiles, two strategies could be suggested such as promoting more growth in Feed 2 condition or enhancing productivity in Feed1 cultures. Condition specific characterization has pointed out that B1 condition were low in culture performance showing low productivity and sudden drop in VCD. On the other hand, A2 condition showed the highest specific productivity and moderate cell VCD therefore leading to the most titer at the end of the culture. Since the combination effect of the basal and feed media is not straightforward, advanced media formulation suggestion should be preceded by comprehension of media effect on cellular metabolism.

4.2 Identify and Compare Culture Media Conditions

To demonstrate the profile-affecting (VCD, titer, SGR and Q_P) impact of multiple metabolites, we implemented MVDA after we converted residual concentration of metabolites into cell specific consumption/secretion rate as preprocessing procedure. Moreover, the data of carbon influx and distribution throughout cellular system, has been calculated for elemental balancing to clarify the rationality of the culture data. Subsequently, PLS was used to determine the most significantly profile-affecting metabolite in spent media of cell cultures. The score plots of PLS accounts for discrimination between culture conditions and their trend is different in *before* and *after feeding*. With multiple metabolites' residual concentration and cell specific rates, it is obvious that basal media type mostly affected the clustering in *before feeding*, but in *after feeding* the difference seems to be resulted primarily from feed types. This result could be evidence for proving the importance of feed media in cell culture especially in later culture, in which the antibody production is active (Figure 3).

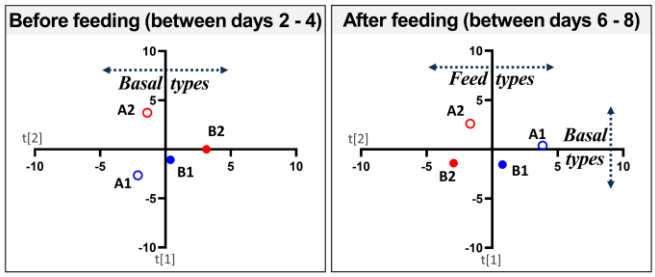

Figure 3. PLS score plots of four combinations of basal and feed media (A1, B1, A2, B2). The model was conducted to determine the effect of specific rates of glucose, amino acids, lactate, and ammonia on specific growth rate.

4.3 Understand CHO Metabolism

Utilizing *in-silico* model (*i*CHO 2291) for further analysis, ecFBA was conducted with calculated specific metabolite uptake and secretion rates. Intracellular fluxes were predicted and significance of basal and feed media effects on metabolic pathways were evaluated (Data not shown). Thus, more subsystems were found to make noticeable flux change according to feed types rather than basal media. Since the network heatmap identifies the importance of feed impact on intracellular flux distribution as well as physiological states, we further investigated the differences in metabolic fluxes on *after feeding* (Figure 4).

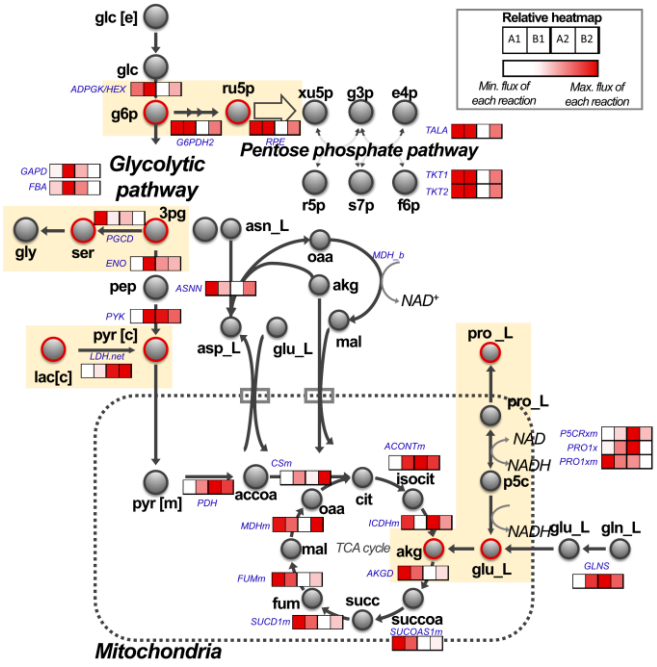

Figure 4. Overall metabolic flux distribution of central carbon metabolism and their side-chain reactions. Simulated intracellular flux distribution under four conditions in *after feeding* phase. To compare the condition-specific fluxes of each reaction, relative heatmaps of single reaction is presented with colored scale from white (minimum) to red (maximum value). Circles with a red and yellow boxes highlighted metabolites and pathways, respectively, which exhibit distinct fluxes.

After flux-based narrow down procedure, it is observed that most of the filtered reactions are participating in glycolysis and TCA cycle while exhibiting condition-specific flux distributions. For example, the reactions in the urea cycle (e.g., mitochondrial ARGN) has high flux on Feed1 conditions (A1 and B1). On the other hand, amino acid metabolisms (e.g., amino acid transferase) are observed that lower flux on Feed1 conditions while the reactions directly and/or indirectly connected to TCA cycle. With the highlighted role of central carbon metabolism under four conditions in *after feeding*, it motivated us to explore and to compare the overall fluxes of the glycolysis, pentose phosphate pathway (PPP), TCA cycle and side-chain reactions. Among the reactions in central carbon metabolism, five pathways (PPP; serine pathway; lactate pathway; TCA cycle; TCA intermediate pathways) have distinct fluxes between Feed1 (A1, B1) and Feed2 (A2, B2). In Feed1, higher glucose uptake and higher fluxes of PPP cause lower utilization of glycolysis. Interestingly, 3pg (3-phosphoglyceric acid) to serine reaction, side-chain reaction of glycolysis (phosphoglycerate dehydrogenase, PGCD), highly activated in A1, while generating more serine within the cell

despite of their lower glycolysis. Moreover, higher consumption of lactate in Feed2 conditions was observed resulting in higher fluxes of citrate synthase under Feed2, allowing the higher flux on TCA entrance reaction. In TCA cycle, we can also observe distinct flux patterns depends on the conditions. Despite the lower flux of TCA entrance flux under Feed1 conditions, the level of TCA cycle intermediates is recovered after α-ketoglutarate (AKG) utilization. Interestingly, only in A1, it generates (AKG) from glutamate through amino acid transferase Here, it should be highlighted that aspartate is the most contributing amino acid in this system. In proline pathway, despite higher proline uptake in A1, most of proline converts into glutamate (P5CDm), while excess amount of TCA intermediate (e.g., AKG) in A2 and B2 makes AKG to generate glutamate.

4.4 Suggest Target Components

In order to enhance specific growth rate of A2, we decided to understand A1 metabolic status which is high growth rate and B1 metabolic status which has poor growth and productivity. Note that we characterized the culture condition of A1 as high growth rate culture, A2 as high productivity culture, and B1 as poor growth and productivity culture. Since our targeting metabolic pathways for A2's growth improvement could be manipulated by feed media components, we evaluated serine and aspartate activity under each condition by quantifying the fluxes of 'glycine, serine and threonine metabolism' and 'alanine and aspartate metabolism' (Figure 5). Serine metabolism, which plays significant role of energy production with serine one-carbon pathway, is observed as differentiated metabolic pathway. Serine metabolism is known to be beneficial for cell proliferation with utilizing serine via glycine hydroxymethyl-transferase (GHMT) in folate cycle, nucleotides synthesis, and generation of NADPH (Labuschagne et al., 2014; Yang & Vousden, 2016). Serine can be supplied by uptake from media or by utilizing glycolysis intermediates (i.e., 3PG) synthesized through one of branched reaction at the late stage of glycolysis. Interestingly, A1 shows high activity on serine system and following methionine and folate cycle. Despite high residual concentration of serine in A1, it showed less uptake of serine, resulting in A1's large conversion from 3PG into serine in order to sustain certain level of flux on GHMT required for cell proliferation. Controlling of serine is required for higher uptake of serine and enhancing flux level of GHMT-coupled reactions in A2.

Glutamate was found to supply TCA cycle intermediate AKG into TCA cycle via glutaminolysis, especially in A1. It is observed that multiple amino acids including aspartate generated or consumed in the glutaminolysis, while aspartate is mostly generated by this metabolism, resulting in enhanced synthesis of TCA intermediates (e.g., oxaloacetate). As for glutamate synthesizing reactions, amino acids such as valine, isoleucine and leucine contribute as substrates more than other amino acids since those amino acids are highly consumed in both A2 and B2. While generating glutamate, generated intermediates are utilized as substrate for synthesizing CoA-intermediates, resulting in their high productivity. Therefore, it is required to supply TCA cycle intermediates with aspartate utilization to enhance cell growth in A2.

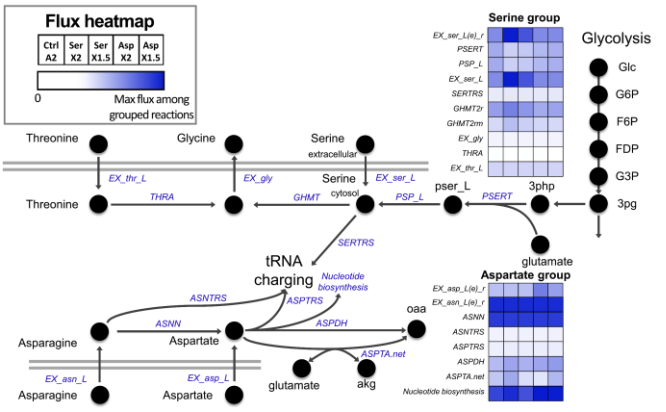

Figure 5. *In-silico* simulation for increased serine and aspartate uptake. Metabolic pathways are presented under five conditions (A2 as control, A2+200% serine, A2+150% serine, A2+200% aspartate, and A2+150% aspartate). Simulated fluxes were illustrated with flux heatmap with colored scale from white (zero flux value) to blue (maximum flux within grouped reactions).

To verify the above-mentioned hypothesis, *in-silico* flux estimation was implemented varying serine and aspartate uptake rates in A2. Here, we conducted verifying procedure with A2 as control to check desired metabolic changes. In Figure 5, serine highly supplemented condition (Serx2) has shown more flux into serine metabolism as well as less carbon source demand from glycolysis via PSERT. More flux into GHMT facilitates serine one-carbon metabolism and it is consistent with increased redox cofactor, especially NADPH which gives a favorable energetic state for better cell growth. Moderate supplementation of serine (Serx1.5) also showed beneficial effects on redox cofactor system. On the other hand, for aspartate, moderate extent of supply (Asp x1.5) led to more generation of TCA intermediate, oxalacetate, facilitating more ASPDH and ASPTA reactions and even supplying more redox cofactors, although it was not detected in high supplementation of aspartate (Aspx2). This indicates adequate amount of aspartate should be supplied for precise complementation for optimum intracellular status. These new insights will be helpful for advanced media formulation strategies.

5. CONCLUSIONS

CHO-K1 cultures with different combinations of basal media and feed additions were selected and each of the culture profile were evaluated. With provided data from experiments, targets of the bottleneck that constraints the culture performance were listed thorough media formulation suggesting systematic data-driven model. Elemental balancing as well as specific exchange rates were calculated to evaluate each culture profiles. Feed2 showed better in production but still limited in cell growth. Therefore, strategies to enhance growth in A2 condition culture were set as objective. For advanced targeting, PLS was implemented to point out the putative targets as serine and proline. Subsequently, ecFBA with CHO GEM was conducted to simulate intracellular flux states and five metabolisms were listed as the bottlenecks. Among them, pathway connections with bottlenecks and metabolites utilization effects were evaluated. Therefore, serine and

aspartate metabolisms were highlighted as target pathways for advanced media formulation. By verifying the effect of each component, serine supplementation was found to guarantee more redox cofactor generation while aspartate addition supplies more carbon source in TCA cycles. Consequently, an advanced media formulation could be suggested by controlling serine and aspartate contents in A2 therefore making potentially beneficial effects on both growth and productivity.

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REFERENCES

Bonarius, H. P. J., Schmid, G., & Tramper, J. (1997). Flux analysis of underdetermined metabolic networks: The quest for the missing constraints. *Trends in Biotechnology*, *15*(8), 308–314. https://doi.org/10.1016/S0167-7799(97)01067-6

Combe, M., & Sokolenko, S. (2021). Quantifying the impact of cell culture media on CHO cell growth and protein production. *Biotechnology Advances*, *50*, 107761. https://doi.org/10.1016/J.BIOTECHADV.2021.107761

Gronemeyer, P., Ditz, R., & Strube, J. (2014). Trends in Upstream and Downstream Process Development for Antibody Manufacturing. *Bioengineering*, *1*(4), 188–212. https://doi.org/10.3390/bioengineering1040188

Hefzi, H., Ang, K. S., Hanscho, M., Bordbar, A., Ruckerbauer, D., Lakshmanan, M., Orellana, C. A., Baycin-Hizal, D., Huang, Y., Ley, D., & Martinez, V. S. (2016). A Consensus Genome-scale Reconstruction of Chinese Hamster Ovary Cell Metabolism. *Cell Systems*, *3*(5), 434–443.

Heirendt, L., Arreckx, S., Pfau, T., Mendoza, S. N., Richelle, A., Heinken, A., Haraldsdóttir, H. S., Wachowiak, J., Keating, S. M., Vlasov, V., Magnusdóttir, S., Ng, C. Y., Preciat, G., Žagare, A., Chan, S. H. J., Aurich, M. K., Clancy, C. M., Modamio, J., Sauls, J. T., … Fleming, R. M. T. (2019). Creation and analysis of biochemical constraint-based models using the COBRA Toolbox v.3.0. *Nature Protocols*, *14*(3), 639–702. https://doi.org/10.1038/s41596-018-0098-2

Hong, J. K., Nargund, S., Lakshmanan, M., Kyriakopoulos, S., Kim, D. Y., Ang, K. S., Leong, D., Yang, Y., & Lee, D. Y. (2018). Comparative phenotypic analysis of CHO clones and culture media for lactate shift. *Journal of Biotechnology*, *283*(August), 97–104. https://doi.org/10.1016/j.jbiotec.2018.07.042

Labuschagne, C. F., van den Broek, N. J. F., Mackay, G. M., Vousden, K. H., & Maddocks, O. D. K. (2014). Serine, but not glycine, supports one-carbon metabolism and proliferation of cancer cells. *Cell Reports*, *7*(4), 1248–1258. https://doi.org/10.1016/j.celrep.2014.04.045

Pan, X., Streefland, M., Dalm, C., Wijffels, R. H., & Martens, D. E. (2017). Selection of chemically defined media for CHO cell fed-batch culture processes. *Cytotechnology*, *69*(1), 39–56. https://doi.org/10.1007/s10616-016-0036-5

Park, S. Y., Reimonn, T. M., Agarabi, C. D., Brorson, K. A., & Yoon, S. (2018). Metabolic responses and pathway changes of mammalian cells under different culture conditions with media supplementations. *Biotechnology Progress*, *34*(3), 793–805. https://doi.org/10.1002/btpr.2623

Rouiller, Y., Périlleux, A., Collet, N., Jordan, M., Stettler, M., & Broly, H. (2013). A high-throughput media design approach for high performance mammalian fed-batch cultures. *MAbs*, *5*(3), 501–511. https://doi.org/10.4161/mabs.23942

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003). Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Research*, *13*(11), 2498–2504. https://doi.org/10.1101/gr.1239303

Sheikh, K., Förster, J., & Nielsen, L. K. (2005). Modeling hybridoma cell metabolism using a generic genome-scale metabolic model of Mus musculus. *Biotechnology Progress*, *21*(1), 112–121. https://doi.org/10.1021/bp0498138

Torkashvand, F., Vaziri, B., Maleknia, S., Heydari, A., Vossoughi, M., Davami, F., & Mahboudi, F. (2015). *Designed Amino Acid Feed in Improvement of Production and Quality Targets of a Therapeutic Monoclonal Antibody*. https://doi.org/10.1371/journal.pone.0140597

Webster, T. A., Hadley, B. C., Dickson, M., Busa, J. K., Jaques, C., & Mason, C. (2021). Feedback control of two supplemental feeds during fed-batch culture on a platform process using inline Raman models for glucose and phenylalanine concentration. *Bioprocess and Biosystems Engineering*, *44*(1), 127–140. https://doi.org/10.1007/s00449-020-02429-y

Yang, M., & Vousden, K. H. (2016). Serine and one-carbon metabolism in cancer. *Nature Reviews Cancer*, *16*(10), 650–662. https://doi.org/10.1038/nrc.2016.81

Yeo, H. C., Hong, J., Lakshmanan, M., & Lee, D.-Y. (2020). Enzyme capacity-based genome scale modelling of CHO cells. *Metabolic Engineering*, *60*(March), 138–147. https://doi.org/10.1016/j.ymben.2020.04.005