Analysis of CHO Cell Batch Culture Data

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Introduction:

Batch culture data on nutrients, product titer and by-products was provided for two Chinese Hamster Ovarian (CHO) cell clones [CHO-TZ and CHO-S7], which were cultivated in two different media [media A and media B]. In this report we aim to visualize, process and analyze this data.

Question 1:

The window for exponential/growth phase appears to be from Day 0 to Day 4, from *Figure 1 (B)*.

During the exponential phase, the primary carbon source is glucose, accompanied by lactate accumulation resulting from aerobic fermentation (*Figure 1 (D)*). However, between Day 4 and Day 6, corresponding to the stationary phase, the cells in Media B appear to utilize both glucose and lactate (*Figure 1 (E)*) as carbon sources. In Media A, the lactate generated during the exponential phase is not re-assimilated by either CHO-TZ or CHO-S7, indicating suboptimal lactate utilization under these culture conditions (This affects our carbon flux to biomass and product).

Within the amino acid (*Figure 1 (C)*), glutamine (GLN) and asparagine (ASN) exhibit sharp declines across all conditions, indicating near-complete consumption irrespective of media or cell line.

Amino acids such as histidine (HIS), isoleucine (ILE), leucine (LEU), lysine (LYS), phenylalanine (PHE), tryptophan (TRP), and valine (VAL) show steady decreases in concentration over the culture period, consistent with the fact that they are essential amino acids.

Serine (SER) displays a gradual but continuous decline throughout the culture period, suggesting it may be a limiting nutrient critical for maintaining cellular metabolism and viability.

The concentration trend of cystine indicates that it is at its optimal concentration in Medium A, as its complete consumption is observed, the difference in cystine concentrations between Medium A and B at the start and end of the process are also nearly the same.

In terms of product formation (IgG titer) as shown in *Figure 1 (F)*, the majority of antibody production occurs during the stationary phase, which is expected since IgG is a secondary metabolite. The final titer profiles display a somewhat counterintuitive trend: for the CHO-S7 cell line, the highest antibody titer is achieved in Medium A, whereas for CHO-TZ, the final titer is higher in Medium B than in Medium A. This further amplifies the idea that media composition plays a crucial role in guiding cellular metabolism and productivity.

Question 2:

The specific rates of the metabolites, specific productivity, and specific growth were calculated for the exponential phase (Day 0–4) using the following relations:

$i)\ \mu = \frac{\ln(X_2) - \ln(X_1)}{t_2 - t_1}$	$ii)\ q_p = \frac{\Delta Titer * 10^6 \left(\frac{pg}{ml}\right)}{Discrete\ IVCD \left(\frac{10^6 (cells * day)}{ml}\right)}$
$iii)\ Specific\ Rate = \frac{concentration \left(\frac{\mu mol}{L}\right) * \left(\frac{10^6 pmol}{\mu mol}\right) * \left(\frac{L}{1000\ mL}\right)}{Discrete\ IVCD \left(\frac{10^6 (cells * day)}{ml}\right)}$	

The calculations are shown in the attached Excel sheet using the exponential-phase data (Day 0–4), and the resulting values are presented in *Table 2*.

Question 3:

Carbon Flux Distribution:

From *Figure 3 (A)*, we see that Carbon flux to biomass takes up the largest fraction, ranging between 6–7 pmol C cell^{-1} day^{-1} across all conditions, with S7-B exhibiting the highest relative biomass fraction (Bio / total C ≈ 0.70).

The high lactate to CO_2 ratios presented in *Table 3 (A)* indicate that a substantial portion of glucose consumed during the exponential phase undergoes aerobic fermentation rather than complete oxidation via the TCA cycle, reflecting less efficient carbon oxidation. Among all conditions, CHO-TZ in Medium B demonstrates the lowest lactate to CO_2 ratio, signifying a more oxidative metabolism with better carbon utilization efficiency.

The antibody fraction of total fixed carbon is highest for TZ-A (2.92 %) and TZ-B (2.93 %), while S7-A (2.17 %) and S7-B (2.26 %) divert comparatively less carbon toward product formation. Although the overall IgG titers of all cultures are relatively close, this analysis indicates that CHO-TZ in Medium B is the most carbon-efficient, achieving a favorable balance between oxidative metabolism and antibody synthesis during the exponential phase.

Carbon Uptake:

From *Table 3 (B)* and *Figure 3 (B)*, glucose remains the dominant carbon source across all conditions, contributing roughly 50–55 % of the total uptake. This is followed by asparagine (ASN), glutamine (GLN), and leucine (LEU).

For a given medium, CHO-TZ exhibits a higher overall carbon uptake, particularly in terms of glucose consumption, compared to CHO-S7.

Looking back to Table 2, the specific growth rate (μ) of CHO-S7 during the exponential phase is significantly lower than that of CHO-TZ (consistent with CHO-S7's slower yet more biomass-oriented metabolism, high relative biomass fraction), which explains its lower glucose uptake values.

The diminished glucose consumption consequently elevates the AA/Glucose ratio observed for CHO-S7, even though both cell lines consume comparable total amounts of amino acids. The higher AA/Glucose ratio in CHO-S7 arises primarily from lower glycolytic activity rather than enhanced amino-acid catabolism.

This highlights the reduced metabolic activity of the CHO-S7 clone, which in turn explains its lower IgG production during the exponential phase relative to the more metabolically active CHO-TZ.

Question 4:

To analyze the specific rates of the different cell lines across different media conditions, a dimensionality reduction was performed using Principal Component Analysis (PCA). In the first section, PCA was applied exclusively to the metabolite-specific rate data to identify underlying metabolic patterns. In the second section, a comprehensive PCA incorporating all specific rates, along with the specific productivity (q_p) and specific growth rate (μ) was performed.

This analysis was performed using JMP software, with the data standardized in both cases to avoid errors arising from the differing units of specific productivity (q_p) and specific growth rate (μ) compared to the metabolite-specific rates.

In the initial analysis PC1 captures 70.8% of the variance in the data, followed by PC2 at 18.6%, PC3 at 10.6%. This is visualized in *Figure 4.1(A)*.

The results of the Principal Component Analysis (PCA) are illustrated through the score plots shown in *Figure 4.1 (B)*. Along Principal Component 1 (PC1), the samples appear to cluster primarily based on the media type, with TZ-A and S7-A positioned closely together, while TZ-B and S7-B nearly overlap along the PC1 axis, indicating strong media-driven metabolic similarities.

Along Principal Component 2 (PC2), the samples appear to cluster according to the cell line, with S7-A and S7-B positioned closely together, while TZ-A and TZ-B almost completely overlap, capturing the similarity in metabolic behavior within each clone regardless of the medium used.

Since these two Principal Components capture our two most essential properties, and also themselves capture 89.4% of the total variance we shall focus on them for further analysis.

From the loading plot (*Figure 4.1 (C)*), it is evident that several amino acids such as histidine (HIS), phenylalanine (PHE), alanine (ALA), isoleucine (ILE), asparagine (ASN), proline (PRO), and leucine (LEU), as well as glucose and lactate (to a slightly lesser extent), exhibit high loadings on PC1. This indicates that these metabolites contribute most strongly to the media-dependent metabolic differences observed between the cultures.

From this plot, it is also evident that glucose and lactate are negatively correlated, suggesting that conditions with higher glucose consumption corresponds to higher lactate production. Additionally, amino acids such as histidine (HIS), phenylalanine (PHE), glutamate (GLU), lysine (LYS), alanine (ALA), isoleucine (ILE), and glycine (GLY) are clustered together, indicating that they exhibit similar metabolic utilization patterns across the different conditions. In contrast, cystine, asparagine (ASN), leucine (LEU), serine (SER), and proline (PRO) form a separate cluster, that is negatively correlated with the first cluster of amino acids, suggesting distinct metabolic behavior among these clusters of amino acids.

Moving onto the second part of the analysis, which includes the specific growth rate (μ) and specific productivity (q_p), it is observed that 90.1% of the total variance is captured by the first two principal components (*Figure 4.2 (A)*). Therefore, these two components were considered sufficient for subsequent interpretation and analysis.

Similar to the previous analysis from *Figure 4.2 (B)*, the first principal component (PC1) primarily captures variation based on media type, with TZ-B and S7-B nearly overlapping along PC1, while TZ-A and S7-A are slightly separated yet remain positioned on the same side of the axis. In contrast, the second principal component (PC2) reflects cell line dependent clustering, where TZ-A and TZ-B group closely together, and S7-A and S7-B similarly cluster along the same axis, indicating distinct clone-specific metabolic behaviors.

From *Figures 4.2 (C) and (D)*, we see that glucose is negatively correlated not only with lactate but also with specific productivity. This indicates that a higher glucose uptake corresponds to both increased lactate secretion and an enhancement in antibody production.

Interestingly, the specific growth rate (μ) shows a positive correlation with proline (PRO), glutamine (GLN), and glucose, while exhibiting a negative correlation with arginine (ARG) and methionine (MET).

The correlations among the clusters of amino acids identified in the prior analysis remains consistent.

Conclusion:

By visualizing and analyzing the batch culture data, we gain a clearer understanding of the performance characteristics of each cell line under different media conditions.

Insights from this analysis enable the fine-tuning of media composition to meet the specific metabolic requirements of the chosen cell line, thereby maximizing overall productivity.
