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A Proposed Study: Chemometric Identification of Canonical Metabolites during Process Parameters Optimization of Chinese Hamster Ovary (CHO) Cell Culture

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Abstract

As the predominated platform of monoclonal antibodies (mAbs) production, it is critical to understand the biological events occur in CHO cells during the manufacturing process. These biological events are often referred as a black box in the industry, and are focusd by many studies due to the recent quality by design (QbD) initiative launched by many regulatory bodies such as FDA and EMA. The QbD efforts are used to understand critical process parameters (CPPs) relevant to its productivity and quality. Many omics studies are used to shine light into this biological black box of CHO cells. However, little study has been done to investigate the biological changes during fermentation by changing of CPPs. These studies are difficult due to large amount of samples and a big set of data. In order to overcome these obstacles, we proposed a dsign of experiment (DoE) to reduce the number of experiments in bioreactor studies to investigate the effect of process parameters (pH, temperature shift and dissolve oxygen (DO)) on protein titer. In this proposed study, pH, temperature shift or DO will be determine if they are CPPs that affect protein titer, and various metabolomic profiles in the bioreactors were also studied. The generated data were analyzed by multivariate data analysis (MVDA) in order to identify the metabolites that were altered by the change of CPPs. The change of DO, pH and temperature in the bioreactor environment lead to significant alternation of metabolites. Therefore, we can speculate the changes of these metabolites lead to titer improvement.

Keywords: Metabolomics; Critical process parameters (CPPs); Recombinant therapeutic protein titer; Chemo metrics; Canonical metabolites

Introduction

Chinese hamster ovary (CHO) cells are one of the most preferred hosts for industrial production of recombinant therapeutic proteins [1]. The performance of CHO cells is usually affected by the extracellular environment that is determined by the process parameters [2]. It is well established that changing of process parameters, such as temperature shift, gas flow rate, dissolve oxygen (DO) and pH, could alter the metabolism of the cells and improve the productivity and quality of monoclonal antibodies (mAbs) [3-5]. Therefore, identifying the key process parameters in manufacturing process is critical to obtain consistent product titer and quality [6]. It also facilitates the understanding of the relationship between the critical process parameters (CPPs) and process output (i.e. productivity, quality etc), which is the key to quality by design (QbD) initiatives by regulatory agencies. Numerous studies are focusing on the bioprocess outcomes, such as viable cell density (VCD), protein productivity and glycosylation [7-9]. However, biological events that are associated with the changes of CPPs have not been wildly investigated [10]. Advance of system biology, such as genomics, proteomics and metabolomics, enables data-driven studies of biological events in a system that is often unclear [11].

Therefore, it provides a solution to understand the biological black box of bioprocess. However, these omics studies of bioprocess are difficult to achieve due to large amount of samples and data generation. To overcome these difficulties, design of experiments (DoE) is needed to limit the samples in the study; and multivariate data analysis (MVDA) is needed to analyze the generated big data set. Therefore, in order to gain insights into the biological events during bioprocess parameters optimization, we propose metabolomics to investigate the biological events during DoE identification of CPPs that affect titer. These data can then analyzed by MVDA to reveal the canonical biological pathways. DoE can significantly reduce the number of experiments required to identify important CPPs while retaining maximum certainty in the effects of the experimental parameters [12]. For bioprocess development, DoE has been successfully applied for identification and optimization of CPPs which are critical for improving cell growth and controlling specific cellular metabolic activity [13-15]. Metabolomics is a common omics method to profile essential nutrients and by-products produced by host cells during bioprocess, and therefore provides insight into the metabolism and canonical pathways. Combining with MVDA, it holds promise to uncover the metabolic characteristics of the optimized process that are critical for both CPPs and titer.

Proposed Experiments

The proposed experimental workflow was summarized in (Figure 1). Briefly, DoE can be set up to identify the CPPs. The metabolic profile of these experiments was determined by liquid chromatography with mass spectrometer (LC-MS). Process parameters that significantly affect the protein titer will be identified the common metabolites identified will be considered to be relevant to both CCPs and titer change, indicating that these metabolites playing critical roles in the improvement of titer when CPPs were altered.

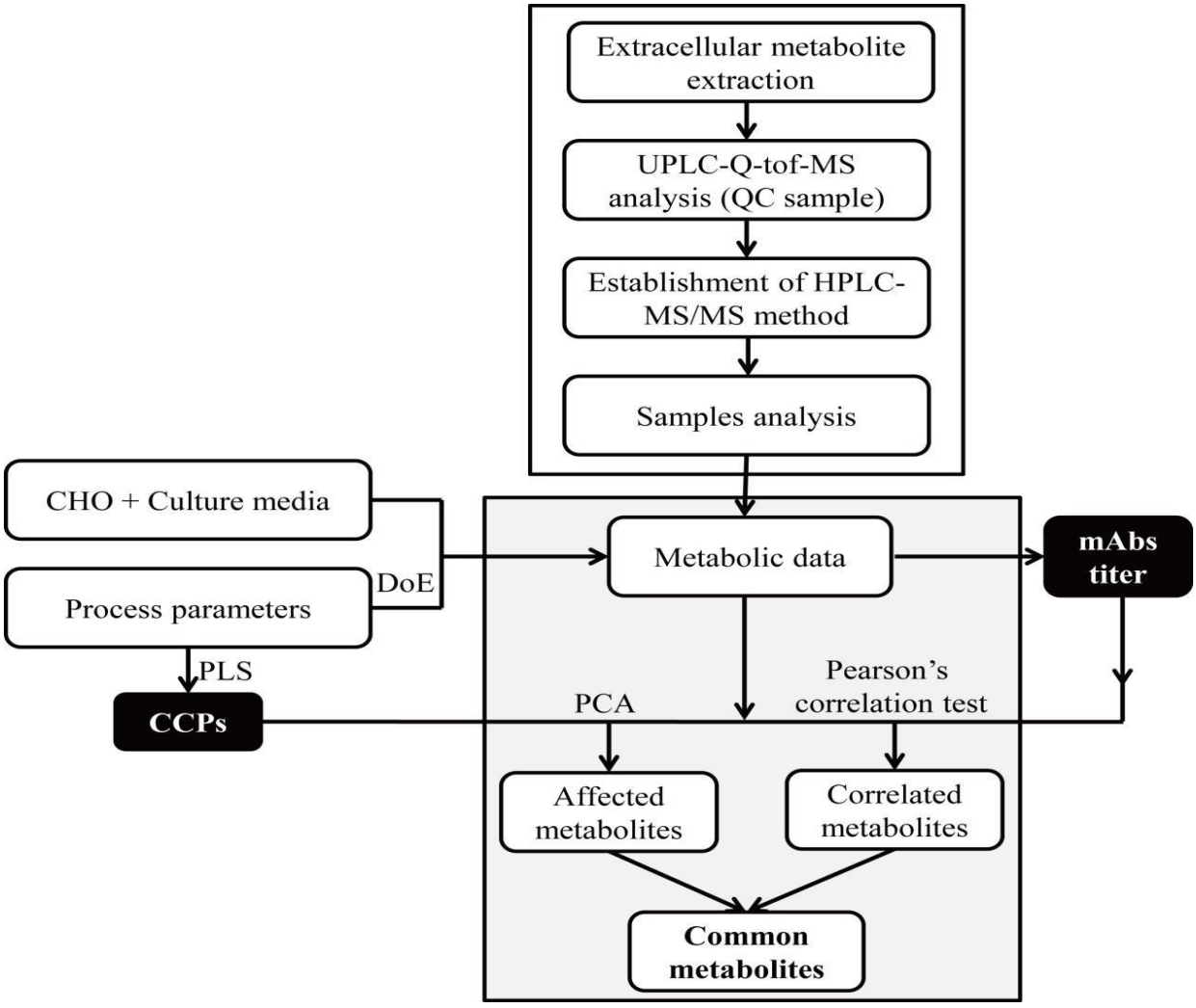

Figure 1: *Workflow overview of current study to identify the metabolites associated with CPPs change and protein titer.*

Metabolites detection and identification

The analyzed metabolites should be focused on amino acids, intermediate products of tricarboxylic acid cycle (TCA), nucleosides and carbon source.

Identify the critical process parameters associated with MABS production

The different process parameters (pH, DO and temperature shift) as X and titer as Y, can be modeled by PLS to identify CCPs. The value R^2Y represented the extracted principal component could sufficiently explain the alteration in Y. The value of Q^2 can show a sufficient predictability of the model. The change of parameters that shows statistical significance of alteration in the process outcomes, will be identified as CPPs for titer.

Metabolic changes during different culture phases

The DoE of process parameters will enable us to observe the alteration of process outcome, and the metabolism and biological events in CHO cells associating with these changes. PCA model can reflect the change of "black box" biological events from the exponential phase to stationary phase, and eventually to apoptotic phase. Within the population of each time point, subpopulations can be identified. For example, samples can be grouped into subpopulations by their pH set values. The subpopulations will indicated that there is difference between the metabolic profile of high titer batches and low titer batches.

Change of metabolism due to PH changes in bioprocess

Since pH may be identified as one of the CPPs that affect titer value at different bioreactor runs, we can further research on the subpopulation in the score plot by different levels of pH values. The samples of bioreactor runs with different pH will be located on different part of the score plot. Separation of subpopulations should be particularly clear in the stationary phase. The score contribution plot, showing which metabolites were influential for the chang of CPP, can be created to identify the metabolites markedly impacted by different levels of pH in different culture phases. For example, metabolites that are involved in transmembrane transport and cell metabolism (such as TCA cycles) can be identified when pH of the culture changed, thus one can speculate that when CHO cells were cultured at different pH, it enabled better transmembrane transport to allow larger amount of amino acids to enter the cells for protein synthesis. The increased consumption of amino acids was previously reported [16].

Change of metabolism due to temperature shift in bioprocess

Temperature shift should be more beneficial for recombinant therapeutic protein production since previous report by Bollati-Fogolín showed that a temperature shift from a 37ºC to 35ºC during stationary phase could increase titer while maintaining high cell density [17]. It was well documented that the temperature shift was routinely used to alter the output of CHO cells cultivated in bioreactors. However, the few researches have been done to investigate the biological events during the temperature shifted. Therefore, a number of potential metabolites will be altered by the 35ºC temperature shift were presented to gain insight into these black box biological events. These metabolites would aid to understand the connection between culture temperature shift and recombinant therapeutic protein productivity.

Change of metabolites associated with titer by pearson's correlation test

The connection between CPPs and recombinant therapeutic protein yield remains as a black-box in bioprocess. The exploration of metabolites can help to gain insights into the potential mechanisms of how CPPs affect protein production. In the above two sections, we can identify series of metabolites impacted by changes of culture pH values and temperature. In this part, the metabolites will show a statistically significant association with protein titer by Pearson's correlation test in different cell phases (Figure 1). Pearson's correlation test will be used to determine the correlation between the concentrations of metabolites and the protein titer values in the bioreactors. The correlation with $P < 0.05$ will be considered as statistically significant. The association between aspartate [16] and protein titer was expected since previous study reported that high concentration of aspartate in medium would inhibit the expression of protein [18]. Nucleosides are the main materials for cellular gene expression. Therefore, gene expression related matabiolite might also involve in the titer improvement during temperature shift. The change of nucleosides in this proposed study will be consistent with previous report by Richardson et al. [19]. Similar to nucleosides, amino acids should also be identified to have a statistical significant correlation with protein titer value. Together with the nucleosides data, we speculated that protein metabolism and gene expression in CHO cells can be activated by the CPPs changes in the proposed study to achieve higher titer in CHO cells. Another canonical pathways can potentially identified by the metabolic study were TCA cycles in cell metabolism. A negative correlation of glutamine attributed to the rapid consumption should be expected as Hong's work did [20]. Malate was involved in TCA cycle for supplying energy during cell culture process. Chong et al also found that malate accumulation throughout the culture process [21]. Lactate was one of the main waste products during

cell culture (Zhou et al. 2011), which should be also accumulated in our proposed study. Therefore, it was speculative that TCA cycles and its metabolites played key roles in CPPs inducing titer change. Combining with the results of PCA, the metabolites linking with pH and temperature shift to recombinant therapeutic protein titer will be all identified the corresponding metabolic pathways of these metabolites are valuable. The biological events linking with the CPPs to protein titer can be identified and illustrated using Reactome Pathway Software. This *in silico* analysis can confirm the metabolic pathways that are sensitive to pH changes. Temperature changes altered the responses of other pathways. Therefore, we speculated that the change of pH and the temperature shift induced the variation of transmembrane transport and metabolism. These changes lead to variation in titer.

Conclusions

By using the state-of-the-art technologies and methods to analyze the CHO cell metabolome during culture, we will be able to gain insight into the biological characteristics during CPPs induced changes in titer. In this proposed work, PLS will be used to identify the CPPs from DoE, PCA and Pearson's correlation test will be applied to interpret the metabolic data to gain valuable information. A series of metabolic makers influenced by pH and temperature shift can be successfully identified. We will find the metabolites that are associated with relevant cell metabolism were key to the titer change when pH change and temperature shift occurred. This facilitated the understanding of the biological events during production process under different conditions and increasing of cell culture knowledge base.

References

1. Wurm FM. Production of recombinant protein therapeutics in cultivated mammalian cells. Nature Biotech. 2004; 22: 1393-1398.
2. Zhang H, Wang H, Liu M, Zhang T, Zhang J, Wang X, et al. Rational development of a serum-free medium and fed-batch process for a GS-CHO cell line expressing recombinant antibody. Cytotech. 2013; 65: 363-378.
3. Dietmair S, Hodson MP, Quek LE, Timmins NE, Chrysanthopoulos P, Jacob SS, et al. Metabolite profiling of CHO cells with different growth characteristics. Biotech Bioeng. 2010; 109: 1404-1414.
4. Qiu J, Chan PK, Bondarenko PV. Monitoring utilizations of amino acids and Vitamins in culture media and Chinese hamster ovary cells by liquid chromatography tandem mass spectrometry. J Pharmaceutical Biomed Analysis. 2016; 218: 163-172.
5. Richardson J, Shah B, Bondarenko PV, Bhebe P, Zhang Z, Nicklaus M, et al. Metabolomics analysis of soy hydrolysates for the identification of productivity markers of mammalian cells for manufacturing therapeutic proteins. Biotech Progress. 2015; 31: 522-531.
6. US FADA. Guidance for industry: quality considerations in demonstrating biosimilarity to a reference protein product. Rockville: US Food and Drug Administration. 2012.
7. Ahn WS, Jeon JJ, Jeong YR, Lee SJ, Yoon SK. Effect of culture temperature on erythropoietin production and glycosylation in a perfusion culture of recombinant CHO cells. Biotech Bioeng. 2008; 101: 1234-1244.
8. Hanson MA, Ge X, Kostov Y, Brorson KA, Moreira AR, Rao G. Comparisons of optical pH and dissolved oxygen sensors with traditional electrochemical probes During mammalian cell culture. Biotech Bioeng. 2007; 97: 833-841.
9. Restelli V, Wang MD, Huzel N, Ethier M, Perreault H, Butler M. The effect of dissolved oxygen on the production and the glycosylation profile of recombinant human erythropoietin produced from CHO cells. Biotech Bioeng. 2006; 94: 481-494.
10. Dean J, Reddy P. Metabolic analysis of antibody producing CHO cells in fed- batch production. Biotech Bioeng. 2013; 110: 1735-1747.
11. Poon F, Mathura VS. Introduction to proteomics. 2009; 107-113.
12. Gruendling T, Guilhaus M, Barner Kowollik C. Design of experiment (doe) as a tool for the optimization of source conditions in sec- esi- ms of functional synthetic polymers synthesized via ATRP. Macromolecular rapid communications. 2009; 199: 589-597.
13. Dong J, Mandenius C, Lübberstedt M, Urbaniak T, Nüssler AK, Knobeloch D, et al. 2008. Evaluation and optimization of hepatocyte culture media factors by design of experiments (DoE) methodology. Cytotech. 2008; 195: 57: 251-261.
14. Lim M, Ye H, Panoskaltsis N, Drakakis EM, Yue X, Cass AE, et al. Intelligent bioprocessing for haemotopoietic cell cultures using monitoring and design of experiments. Biotech Advan. 2007; 25: 353-368.
15. Mandenius CF, Brundin A. Bioprocess optimization using design - of - experiments methodology. Biotech Progress. 2008; 24: 1191-1203.
16. Yoon SK, Choi SL, Song JY, Lee GM. Effect of culture pH on erythropoietin production by Chinese hamster ovary cells grown in suspension at 32.5 and 37.0 C. Biotech Bioeng. 2005; 89: 345-356.
17. Fogolín M, Forno G, Nimtz M, Conradt HS, Etcheverrigaray M, Kratje R. et al. Temperature reduction in cultures of hgm - csf - expressing cho cells: effect on productivity and product quality. Biotech Progress. 2005; 21: 17-21.
18. Kou T, Fan L, Zhou Y, Ye Z, Zhao L, Tan W. Increasing the

productivity of TNFR-Fc in GS-CHO cells at reduced culture temperatures. Biotech Bioproc Eng. 2011; 16: 136-143.

19. Richardson J, Shah B, Bondarenko PV, Bhebe P, Zhang Z, Nicklaus M, et al. Metabolomics analysis of soy hydrolysates for the identification of productivity markers of mammalian cells for manufacturing therapeutic proteins. Biotech Progress. 2015; 31: 522-531.
20. Hong JK, Cho SM, Yoon SK. Substitution of glutamine by glutamate enhances production and galactosylation of recombinant IgG in Chinese hamster ovary cells. Applied Microbiol Biotech. 2010; 88: 869-876.
21. Chong WP, Reddy SG, Yusufi FN, Lee D, Wong NS, Heng CK, et al. Metabolomics-driven approach for the improvement of Chinese hamster ovary cell growth: overexpression of malate dehydrogenase II. J Biotech. 2010; 147: 116-121.
22. Zhou M, Crawford Y, Ng D, Tung J, Pynn AF, Meier A, et al. Decreasing lactate level and increasing antibody production in Chinese Hamster Ovary cells (CHO) by reducing the expression of lactate dehydrogenase and pyruvate dehydrogenase kinases. J Biotech. 2011; 24: 27-34.