

Streamlined Strategy for Rapid Development and Optimization of Cell Culture Media Supporting High Recombinant Protein Production in Chinese Hamster Ovary Cells

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Introduction

The robustness of Chinese Hamster Ovary (CHO) cells in large-scale recombinant protein production and their ability to sustain active biological function of expressed proteins prompts their wide usage in pharmaceutical companies. However, recombinant CHO cells are challenging to optimize because of the diverse nutritional needs unique to every clone.

It is essential to develop the most efficient and optimal media development strategy possible. The traditional approach frequently used in the industrial setting is to test one component at a time to determine its optimal level, which is labor intensive, costly and time consuming. SAFC Biosciences' CHO cell culture platform includes high-throughput screening technology, a diverse selection of CHO media (both animal-component free and chemically defined formulations), state-of-the-art cell engineering technology and statistical Design of Experiment (DOE) design software; the platform allows us to make CHO media optimization an extremely efficient process.

In this presentation, we will introduce the new streamlined strategic approach to speed media development by applying the "CHO Media Library", DOE media mixing screening, spent medium component analysis and bioprocess cell feeding strategies. With this strategy, compared to the traditional method, we can analyze multiple criteria simultaneously to determine synergistic responses. We can then identify one or more media mixtures that provide the desired user outcomes. Meanwhile, an example will be used to depict use of this strategy to efficiently develop optimized formulations for a humanized IgG-producing CHO clone.

Materials and Methods

Cell lines and media:

Table 1: shows the CHO cell lines tested with CHO Media Library. The stock cultures of these cells were cultured in EX-CELL™ CHO DHFR- Medium, an animal-component free formulation (Item No. C8862 or proprietary formulations). The stock cultures were pre-adapted to the test formulations or directly seeded into the test formulations without adaptation. All SAFC Biosciences formulations and two competitor media were supplemented with 6 mM L-Glutamine unless otherwise specified. The data for this presentation was from 125 mL shaker flask culture.

Cell Line	Cell Line Lineage	Recombinant Protein Produced
CHO-K1	CHO-K1 parental	None
CHO-S	CHO-S parental	None
CHO-AP1	CHO-K1 derived	Alkaline Phosphatase
CHO-AP2	CHO-K1 derived	Alkaline Phosphatase
CHO-hGH1	CHO-K1 derived	Human Growth Hormone
CHO-hGH2	CHO-K1 derived	Human Growth Hormone
Recombinant CHO line 1	CHO-K1 derived	Human IgG
Recombinant CHO line 2	CHO-S derived	Human IgG

Table 1. CHO Cell Line Models for CHO Media Library Study

Analytical methods and productivity assays:

Alkaline phosphatase activity: alkaline phosphatase activity was measured with Alkaline Phosphatase Fluorescence Detection Kit according to the manufacturer's protocol (Sigma-Aldrich, AP-F).

Human growth hormone: hGH productivity was quantified with hGH ELISA Detection Kit (Roche). The procedures were conducted at the manufacturer's protocol with minor modifications.

Human IgG: IgG concentration was measured with Protein A affinity chromatography.

Statistical analysis:

In the DOE mixing experiment, User-Defined Design category was used to generate the mixing combinations to make a "Pyramid" model (Figure 2). The DOE screening data was analyzed by Design-Expert® Version 7.0.1 (Stat-Ease).

Results and Discussion

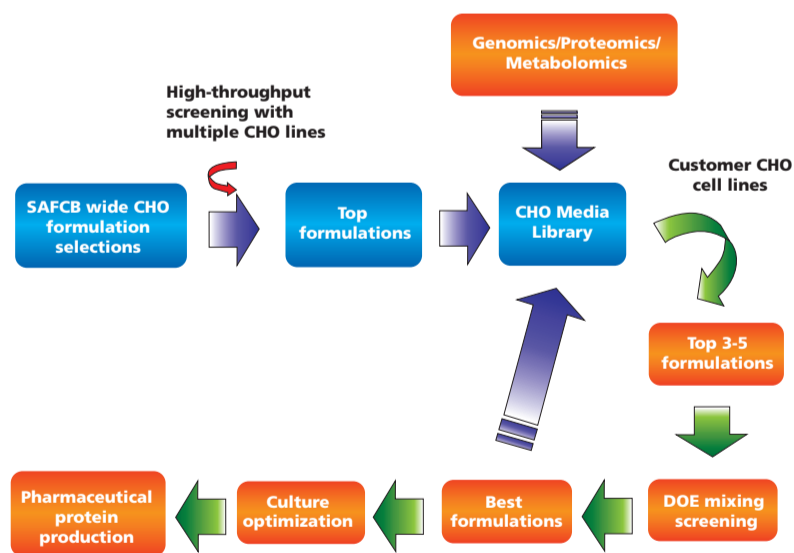


Figure 1. Schematic representation of the strategic platform for CHO media development

To develop the CHO Media Library, multiple CHO cell lines were tested against a wide selection of CHO formulations from SAFC Biosciences. Top formulations were ranked based on cell growth and protein productivity. Customer CHO cell lines will be screened with the CHO Media Library and the top 3 – 5 formulations will be selected for DOE mixing screen to identify the best formulation mixtures supporting the customer cell lines. After optimization of cell culture conditions (e.g. temperature shift and cell feeding), the selected formulations will significantly improve the pharmaceutical protein production in an efficient manner. Meanwhile, the selected top formulations and SAFC Biosciences' ongoing state-of-the-art biology studies (Genomics/Proteomics/Metabolomics) will continue enriching the contents of CHO Media Library.

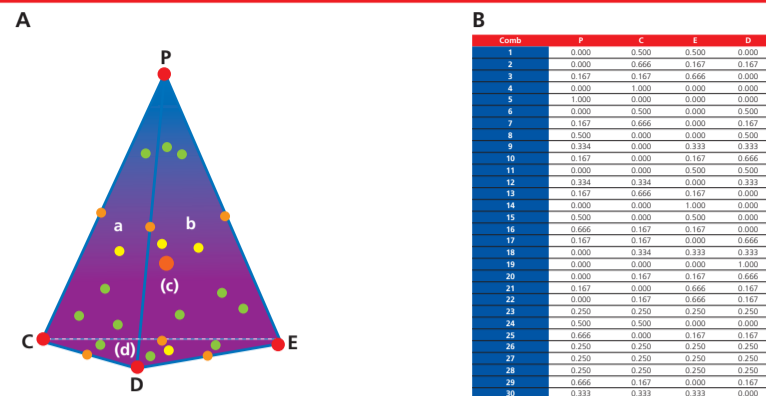


Figure 2. DOE "Pyramid" Design

A) Schematic representation of DOE design. Four SAFC Biosciences' formulations (C, D, E and P) have been chosen for DOE mixing analysis. To simplify the experimental design, only the combinations on the surfaces (a-d) of pyramid have been evaluated, including vertices points (red spots), centers of edges (orange spots), surface centroids (yellow spots), axial check blends (green spots) and overall centroid (big red spot)

B) DOE combination table (including 26 combinations and 4 replicates of overall centroids).

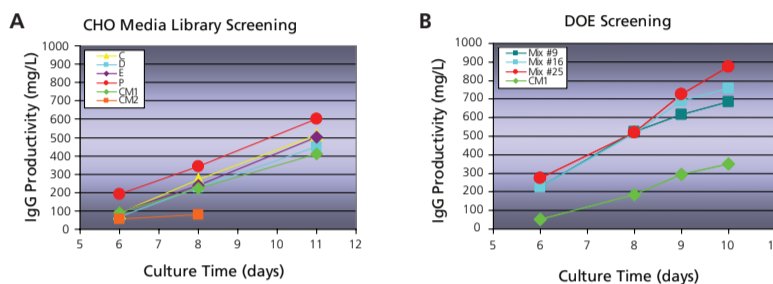


Figure 3. IgG Productivity Comparison

A) IgG production in selected formulations from CHO Media Library along with 2 competitor media.

B) IgG production in selected mixing formulations after DOE screening along with competitor medium A. DOE mixing dramatically improved the IgG productivity ~ 2-fold higher than that in competitor medium A.

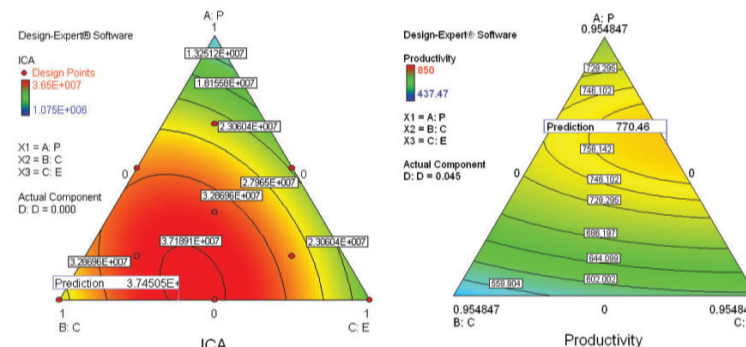


Figure 4. DOE Statistical Analysis and Numerical Optimization

A) DOE optimization based on the cell growth (ICA: specific cell growth). It can be observed that more of formulation C would be beneficial to improve cell growth.

B) DOE optimization based on IgG productivity. More of formulation P would be beneficial to improve IgG production. Therefore, based on the features of the specific cell lines, the different mixing strategies will be applied to fit the needs.

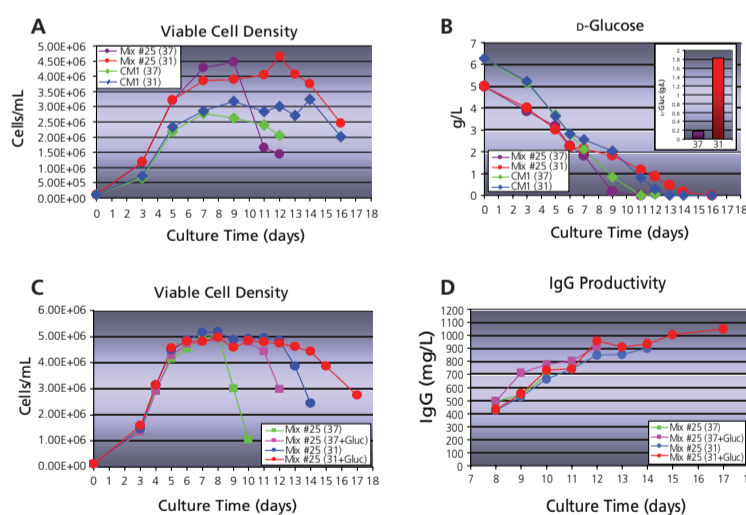


Figure 5. Cell culture condition optimization with Mix #25

A) The cell growth under temperature shift study. The cell cultures were switched from 37 °C to 31 °C on day 6.

B) L-glucose consumption rate has been significantly slowed down in 31 °C cultures. The inset compares the L-glucose level in the Mix #25 with the two temperatures on day 9

C) The cell growth under the temperature shift and L-glucose feeding conditions in the Mix #25

D) IgG productivity in Mix #25 under the temperature shift and L-glucose feeding condition.

Conclusions

- SAFC Biosciences' cell culture platform (high-throughput screening, diverse selections of CHO media, cell line engineering, DOE design and systems biology methodologies) facilitates the new strategic approach to speed media development. In comparison with traditional development approaches, use of this platform dramatically reduces costs and development time (usually less than 5 months).
- In this study, we first present the four-component DOE analysis (pyramid model) which turns out to be more informative and efficient than the conventional three-component DOE analysis (triangle model).
- With an IgG-producing CHO clone, the CHO Media Library platform has developed several well-performing formulations supporting IgG production, Mix 25 demonstrates the efficacy of this platform by nearly tripling the IgG productivity as compared to the control (competitor A) after culture modification (temperature shift).