An Efficient Approach to Cell Culture Medium Optimization — a statistical method to medium mixing

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Abstract

One of the most challenging aspects of culturing recombinant Chinese Hamster Ovary (CHO) cell clones is providing for the diverse nutritional requirements that are unique to every transfected cell line. In order to minimize the amount of time required for medium development, we have recently developed CHO Medium Optimization Kit 1 (Product Code CH0001). This kit consists of six diverse animal component-free CHO media (including two chemically defined media) to provide for a wide range of nutritional requirements. This CHO medium optimization kit provides a convenient format for screening multiple media, but also functions as a platform for statistical medium optimization by using a three-point mixing design. After screening several different recombinant CHO clones against the six media, our data showed that we could increase cell growth and recombinant protein production over the original medium. Following a statistical approach provided by Design-Expert® computer software, we mixed the top three performing media and were able to further increase cell growth and productivity. The data can be further analyzed by Design-Expert® to yield a predicted best medium mixture to support maximum cell growth and productivity for a particular recombinant CHO clone. Taken together, our data strongly suggests that using CHO Medium Optimization Kit 1 with a combination of media screening and a statistical approach to media mixing, can facilitate the development of an optimized medium for any given recombinant CHO clone.

*Design-Expert® is a registered trademark of Stat-Ease, Inc.

Introduction

Chinese Hamster Ovary (CHO) cells have been extensively used for recombinant protein production by many researchers in both academics and industry. Different CHO cells have been shown to exhibit a large degree of variability when it comes to their nutritional requirements. As a result, every transfected CHO cell line can have a unique set of nutritional requirements for maximizing cell growth and recombinant protein production. It is therefore essential to be able to develop an optimal medium formulation in the most efficient manner possible. This need has led to the development of CHO Medium Optimization Kit 1.

Sigma's CHO Medium Optimization Kit 1 consists of six diverse animal component-free media designed to maximize cell growth and recombinant protein production in a wide variety of CHO clones. Two of these six media are chemically defined and all differ in amounts of amino acids, vitamins, salts, trace elements, recombinant human insulin and other organic compounds. The format of this kit allows the user to rapidly screen six CHO formulations for cell growth, recombinant protein production or any other criteria. If this initial screen yields satisfactory results, the researcher may decide that no further optimization is required. However, if the initial screening does not satisfy the specified criteria, a series of media mixing assays can be performed.

Once the media mixing assays have been completed, there are two options for data analysis. The first option is to visually analyze the data for each criterion. This can be accomplished by comparing raw or normalized data for cell growth kinetics, recombinant protein production and any other criteria. The second method for data analysis is a more in-depth approach, in which a Design-of-Experiment (DOE) software package can be used. The DOE software can analyze the media mixing data and allow the researcher to assign importance values to each criterion. Based on these inputs, mathematical models are then used to predict the outcome of an infinite number of combinations of the three media and the desirability of each mix. The final outcome is one or several best-fit media designed specifically to meet the nutritional requirements of a particular cell line.

As more and more recombinant CHO clones have been developed, it has become increasingly important to streamline the medium optimization process. The convenient format of CHO Medium Optimization Kit 1 allows for a rapid screening of multiple diverse CHO formulations. In addition, the powerful mixture experiments coupled with the DOE software provide an invaluable tool for boosting cell growth and productivity. In either application, CHO Medium Optimization Kit 1 has the ability to significantly improve any medium development project.

Materials and Methods

Sigma-Aldrich Corporation (St. Louis, MO) supplied all chemicals, media and solutions unless otherwise stated.

Cell Lines

CHO K1 cells (ATCC # CRL-61) were obtained from the American Type Culture Collection (ATCC). Cell line CHO IgG, expressing a proprietary recombinant antibody, was transferred from a customer to Sigma for custom medium development and optimization.

Culture Media

The media used in this study are CHO Protein-Free Animal Component-Free Medium.

Cell Culture and Cell Growth Assays

Cells were grown in suspension in #C5467 and were used to seed experiments done in 125 mL or 250 mL (100 mL and 150 mL liquid volume respectively) Techne Spinner flasks. The initial innoculum was 50,000 viable cells per milliliter. The cells were cultured in Forma incubators at 37 °C and 5% CO_2.

Spent medium samples were collected every day for the analysis of nutrients/metabolites and recombinant protein production. At the same time, the cells were counted using a Schärfe System Casy 1® Model TTC and viability was assessed using the Trypan Blue Exclusion Method.

Quantification of Recombinant Humanized IgG

The IgG secreted into the medium by CHO IgG was measured by HPLC (Waters 2690 HPLC Millipore, MA) using a protein-G affinity column.

Results

Components of CHO Kit 1

Product code	Description of Medium
C 5467	CHO Medium, Animal Component-Free
C 8862	CHO DHFR-Medium, Animal-Component Free
C 4726	CHO Medium, Chemically Defined, Animal Component-Free
C 9737	CHO Medium 4, Animal Component-Free
C 0363	CHO Medium 5, Animal Component-Free
C 0488	CHO Medium 6, Chemically Defined, Animal Component-Free

Table 1. Complete media selected as components of CHO Medium Optimization Kit 1. After screening several recombinant CHO clones against our library of CHO media, we found each cell line had a unique set of nutritional requirements. The above media were chosen based on chemical diversity and performance for cell growth and recombinant protein production.

Initial Screen of CHO Medium Optimization Kit 1

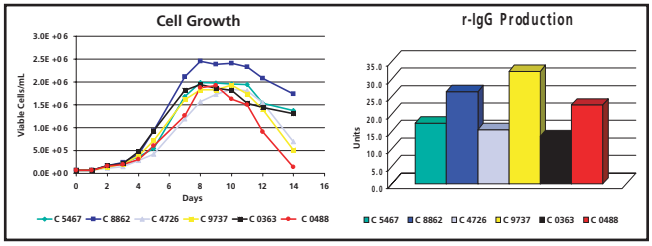

Figure 1. Examining cell growth and productivity after the initial screening of all six media. The first step of any mixtures assay is to screen all six media to determine which media perform best for each CHO clone. Cell growth and protein production for a recombinant CHO clone are depicted in Figure 1. For cell growth, C8862 performed better than all other media. However, this clone produced the most r-IgG in C9737.

Mixtures Triangle with Respective Media Mixes

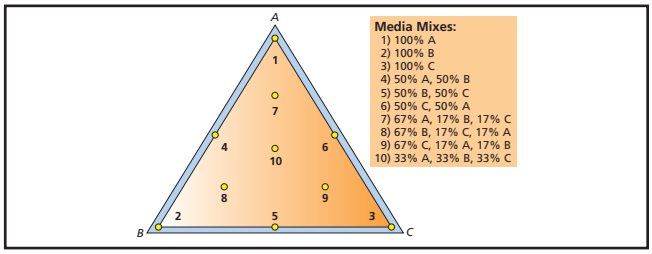

Figure 2. The three-point mixtures diagram and corresponding media mixes. The three best performing media from the screening of all six media, are set to 100% at one of the three vertices of the mixtures triangle. Media mixing begins at 50% between two of the media along the sides of the triangle (numbers 4, 5 and 6). This is followed by a 67%, 17%, 17% mix of all three media within the interior of the triangle (numbers 7, 8 and 9). The final mix will be 33% of all three media as seen at the center point of the triangle (number 10).

Cell Growth and Productivity from the Mixtures Experiment

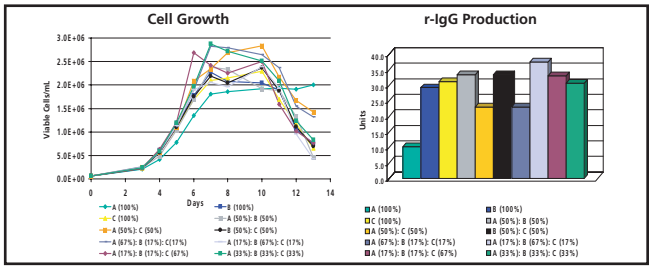

Figure 3. Cell growth and productivity for a recombinant IgG producing CHO clone were compared following the mixtures experiment. The media represented here are as follows: Medium A = C5467, Medium B = C8862 and Medium C = C9737. The data reveals that practically every mix performed better for cell growth. In addition, these media mixes correlated with the highest levels of recombinant protein production. This data can be analyzed by using two separate methods as indicated in the following figures.

Data Analysis by Comparing Normalized Data

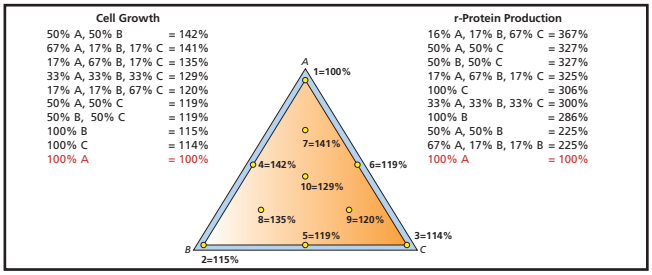

Figure 4. Data from the mixtures experiment can be normalized and visualized in several ways. The data from the mixtures experiment in Figure 3 can be analyzed by normalizing the data to one of the media. In this example, the results from medium A represent 100%. Media can be ranked in terms of performance in the form of a list as seen on the left and right of the triangle. Another way of visualizing the data is to plot the values on the mixtures triangle. The center diagram represents the normalized cell growth data. This method clearly indicates an increase in cell growth for the media mixes around numbers 4, 7, 8 and 10.

Using DOE Software for a More Precise Data Analysis

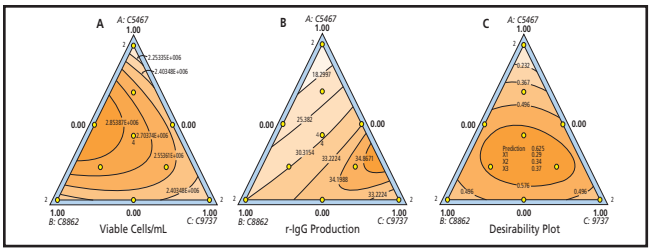

Figure 5. Data from the mixtures assay can also be analyzed using Design-of-Experiment (DOE) software for a more precise analysis and optimization. A.) The contour plot for cell growth data indicates that the area with the best cell growth is located near a 50% mix between C8862 and C5467. B.) A similar contour plot for recombinant protein production. C.) During the optimization step, importance values can be assigned to each criterion, allowing each criterion to be weighted separately or together. The software then generates a desirability plot with values ranging from 0 to 1 (1 being the most desirable), indicating the exact point on the triangle where the most desirable mixes are located. *The point predicted to yield maximum cell growth and r-IgG production corresponds to a mix of 29% C5467, 37% C8862 and 34% C9737.*

The Recommended Media Mixes

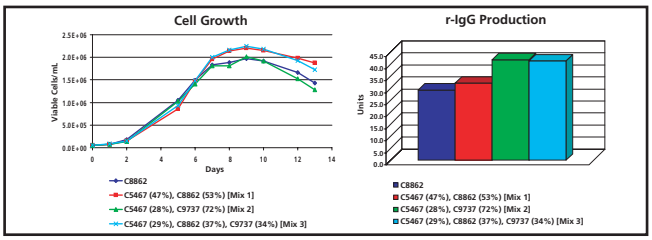

Figure 6. The media mixtures recommended during the optimization step were tested. The mix predicted to give the best cell growth and was located on the left side of the mixtures triangle at 47% C5467 and 53% C8862 (Mix #1). The mix predicted to have the best productivity and was located on the right side of the mixtures triangle at 28% C5467 and 72% C9737 (Mix #2). When we assigned equal importance values to growth and productivity, the mix with the best combined cell growth and productivity was in the middle of the triangle at 29% C5467, 37% C8862 and 34% C9737 (Mix #3). The above figures show that the media mixes indicated to yield the best cell growth and production performed as predicted.

Discussion

Sigma's CHO Medium Optimization Kit 1 (Product Code CH0001) was developed to provide researchers with a tool to increase the efficiency of CHO media optimization. Included in this kit are six diverse media (see Table 1) designed to meet the wide range of nutritional requirements seen in CHO clones. These six formulations were selected from our media library after screening them against several recombinant CHO cell lines.

Figure 1 shows the initial screening of all six media. After the initial screening, the user must decide whether the results are sat-

isfactory, or if additional optimization is desired. Based on criteria such as cell growth and recombinant protein production, the user can select the three best performing media and complete a mixing assay. Figure 2 introduces the concept of the mixture triangle and the media mixes corresponding to each point on the triangle.

The top three performing media from the initial screening experiment shown in Figure 1 were used to perform a mixing experiment. The results from this mixing experiment are depicted in Figure 3. Not only did the mixes increase cell growth, but they also correlated with the highest levels of recombinant protein production. Data analysis for this assay can be done by two methods. The first method, represented in Figure 4, is done by separately analyzing the data for each criterion. The data for cell growth and recombinant protein production can be normalized to one of the media and ranked in order to make a comparison for each criterion. This normalized data can also be visualized by directly plotting it on the mixture triangle. Using this method allows the researcher to identify which area of the triangle might reveal the best possible mixes.

If the user desires a more in-depth analysis, a Design-of-Experiment (DOE) software package such as Design-Expert® (Stat-Ease, Inc.) can be used. By using DOE software, it is possible to find one or several best-fit media for a particular CHO clone. The software accomplishes this by using mathematical models to predict the outcome of an infinite number of combinations of the three media selected. One unique feature of this program is that specified criteria can be assigned importance values based on the user's needs and desired outcomes. Design-Expert® can then identify one or several optimal media mixtures that will provide the user with the best medium possible. Another major benefit of this method for data analysis, is that multiple criteria can be analyzed together to determine synergistic responses between the criteria.

Figures 5A and 5B show the contour plots generated for our two criteria, cell growth and recombinant protein production. After an importance value has been assigned to each criterion, Design-Expert® will generate one or several optimal media mixes that are predicted to meet the desired outcome. Figure 5C is a graphical representation of the desirability plot for all possible mixes that can be formulated from the three media. The desirability ranges from 0 to 1, and indicates how the predicted mixtures might perform. In our experiment we assigned equally high importance values for both criteria. The result is a predicted medium mixture that will produce the maximum cell growth and recombinant protein production for our particular clone. If we alter the importance values, the desirability plot will change to reveal a different best-fit medium mixture.

The final step in this medium optimization process is to test the recommended media mixtures. Figure 6 depicts the three media mixtures that Design-Expert® recommended based on the importance values that were assigned to each criterion. The results show that Mix 1 did increase growth as predicted, Mix 2 did enhance productivity as predicted, and Mix 3 performed the best for both cell growth and productivity. By using the media and techniques described in CHO Medium Optimization Kit 1, it is possible to develop an optimized medium for any CHO clone. The data presented here has confirmed the value of this approach to medium development.

Conclusions

- Sigma's CHO Medium Optimization Kit 1 consists of six diverse animal component-free media formulations designed for maximum cell growth and recombinant protein production in CHO cells.
- Convenient format provides multiple media formulations that can be screened for any recombinant CHO clone.
- Simple yet powerful media mixing experiments combined with DOE software allow researchers to rapidly elucidate an optimal medium mixture.
- Any of the optimized media mixes generated for a particular recombinant CHO clone can be manufactured by our GMP or GLP facility.

Acknowledgements

We would like to thank everyone who contributed to the research and development of CHO media at the Sigma-Aldrich Saint Louis R&D facility.