Methods of Rational Culture Media Design and Optimization for a Hybridoma and a Myeloma Cell Line

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Abstract

Optimizing a chemically-defined cell culture medium is a challenging task and can be broken down into three basic phases: screening, optimization, and verification. For rapid and efficient medium optimization, it is critical that each of these phases is accomplished using the most suitable methods chosen from the large variety available. In this project, we will show two of the many methods we employ for rational design of serum- and animal component-free media for hybridoma and myeloma cell lines. In the first screening phase, a traditional blending methodology utilizing a rapid metabolic assay in 96-well plates was used to find prospective prototype formulations providing better cell growth performance of the hybridoma cell line (HFN) than the initial prototype medium. HFN cell growth in 3 prospective formulations was compared at the shaker flask scale; the best of these formulations gave about 3x better performance than the initial prototype medium and was chosen for further optimization. An IgG expressing NS0 myeloma cell line also grew well in the new prototype medium and was also used in optimization-phase screening experiments. A design of experiment (DoE) screening methodology was used to perform experiments with both cell lines to identify component groups in the prototype medium having the greatest effect on cell growth and productivity and those groups that interact with others. Our experiments show that for HFN cells, amino acid and nutrient groups had the largest effects on both cell growth and productivity while for NS0 cells, amino acids had the largest effect on cell growth, but both amino acid and nutrient groups had large effects on productivity. For comparison, these two groups were then optimized for HFN cells using both traditional titration and design of experiment surface response techniques. The first round of DoE surface response experiments gave optima of 1.27x for the amino acids and 0.37x for the nutrient group. Traditional titration of the two groups gave optima of 1.54x for the amino acids and 1.02x for the nutrients. Verification experiments are underway to determine the method giving the best optima for these groups. These results highlight the need for multiple methods of culture medium design and optimization, that different cell lines have different nutritional needs and that verification experiments are necessary at each step in the culture medium optimization process.

Introduction

Optimization of the industrial production of a recombinant antibody or other protein is accomplished by optimizing the culture medium, cell line, and production processes. A given cell culture medium consists of a large number of components and each cell line has specific requirements for optimal growth and productivity of its recombinant protein of interest. Developing an optimized medium for a cell line is a challenging and complicated process and is best accomplished using a variety of methods chosen for suitability at each step of optimization from the large variety of available methods. Traditional optimization methods, such as titration of individual components, are reliable but can be labor intensive and time consuming when evaluating the effects on cell growth and productivity of numerous factors or groups of factors. It is also difficult to evaluate interactive effects of various media components with traditional optimization methods. Design of experiment methodology (DoE) is useful in some optimization steps to reduce the number experiments and allow discovery of media component interactions while keeping the size and scope of experiments at a manageable level. In this study we use traditional and DoE methods to rapidly optimize groups of components in media optimization for a hybridoma and myeloma cell line. A traditional media blending method was used to find a well performing initial prototype medium . To optimize the prototype medium, DoE screening studies were used to determine component groups with the greatest effect on cell growth and productivity of the model cell lines. Then surface response methods were used to further optimize those groups. Due to the discrepancies found between surface response and titration experiments, evaluation and verification studies are currently being conducted to determine the best method for component group optimization.

Methods

Cell lines
HFN 7.1 (HFN) cells producing anti-human fibronectin IgG from American type culture collection (ATCC #CRL-1606) and a NS0 cell line expressing a recombinant monoclonal antibody were used in these studies. They were both adapted to serum-free media and grown in chemically defined media.

Culture
Cells were grown in stationary 96-well plates or 125mL shaker flasks on an orbital shaking platform at 37°C under 5% CO_2. Cells were seeded at 2 x 10^5 cells /mL, and viable cells/mL was measured using Vi-Cell™ cell viability analyzer (Beckman Coulter, USA) on each day. The IgG concentration in the medium was determined by HPLC using a size-exclusion column and UV absorbance detection at 280nm.

Blending Experiments
Blending experiments were conducted in 96 well plates with 8 replicate wells per blend. Duplicate plates were inoculated and assayed for metabolic activity on day 0 (inoculation) and day 3 (cell growth) using the Alamar Blue Assay (Biosource Int'l, pn DAL1100).

Design of Experiment Screening and Surface Response Experiments
Design of experiment methods and analysis were determined using ECHIP software version 6 (ECHIP, inc.). Briefly, cell culture medium was divided into a number of component categories (amino acids, vitamins, etc.). Group concentrations and combinations for each design point medium were selected by the software based on the concentration limits given and experimental approach chosen. All media were adjusted to the same pH and osmolality prior to inoculation with cells.

Results

Case Study 1: A Well-Performing Prototype Medium Was Found Using A Traditional Media Blending Experiment

Growth of HFN hybridoma cells was compared in all combinations of blends of 15 serum-free and traditional cell culture media to that of our initial hybridoma prototype medium 0 (Hyb prot 0). One panel of 6 is shown in figure 1A. Cell growth performance in 96-well plates was evaluated using the Alamar Blue Assay with 8 replicates per sample. Blends showing best metabolic activity on day 3 (identified with blue circles) were tested at the shaker flask scale in Figure 1B & C. Both IVC (Fig. 1B) and cell growth curves (Fig. 1C) were used for comparison. Blend B gave over three-fold better cell growth performance than the Hybriboma prototype 0 medium and was assigned as Hybridoma prototype 1 for future optimization experiments.

Figure 1A. Metabolic Activity of HFN Cells on Day 0 (inoculation) and Day 3 (growth).

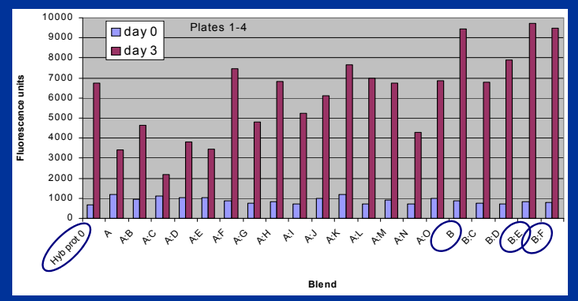


Figure 1B. IVC (Integral of Viable Cells) of HFN Cells grown in different media blends.

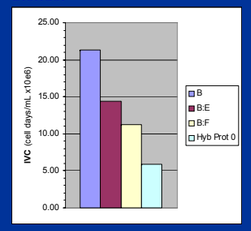
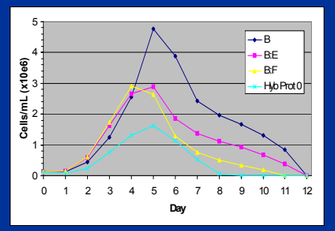


Figure 1C. Growth Curves of HFN Cells grown in different media blends.



Case Study 2a: Screening of Medium Component Groups Detected Differences in Nutritional Needs in Different Cell Types

Design of Experiment Screenings using the 'linear with center-point methodology' (Table 1) were conducted to determine which of 4 groups of media components from hybridoma prototype 1 had the greatest effect on cell growth (Fig. 2A) and productivity (Fig. 2B). Two cell lines were used for comparison and were HFN 7.1 hybridoma and an IgG expressing NS0 myeloma cell line. The four variable component groups were: Amino Acids, Nutrients, Vitamins, and Trace Metals. A fifth group was kept constant and was comprised of salts. For the HFN cell line, the amino acid and nutrient groups had the greatest effect on both cell growth and productivity (Table 2). For the NS0 cell line, amino acids had the greatest effect on cell growth while amino acid and nutrient groups had the greatest effect on productivity (Table 2).

Case Study 2a (continued):

Table 1. Linear With Center Point Screening Experimental Design. There are 22 design points with 8 replicate design points (23-30). Values given for each media group are ratios of the amounts in the hybridoma prototype 1 formula.

Design Point	AA	Nut	Vit	TM
1 23	0.25	0.25	0.1	0.1
2 24	1	0.1	0.25	0.1
3 25	0.1	1	1	0.1
4 26	1	1	0.1	0.25
5 27	0.1	0.25	0.25	1
6 28	0.25	0.1	1	5
7 29	2.5	2.5	2.5	10
8 30	5	5	5	0.25
9	5	1	2.5	1
10	1	5	2.5	5
11	2.5	5	1	1
12	2.5	1	5	5
13	5	2.5	0.1	5
14	1	2.5	5	1
15	0.25	5	0.25	10
16	1	0.25	1	10
17	0.25	2.5	2.5	0.25
18	2.5	0.25	0.25	0.25
19	5	2.5	0.25	0.1
20	2.5	0.1	0.1	1
21	0.1	0.1	0.1	0.1
22	5	5	5	10

Table 2. Summary Of Results By ANOVA Analysis. IVC and Productivity are the response variables. The star indicates a statistical significance. Both amino acids and nutrient groups have greater effects on cell growth and productivity. The number of stars (1-3) indicate increasing significance of a groups effect on a given response. A (.) indicates no significant effect.

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HFN_IVC
.    HFN_prod
.    .    NS0_IVC
.    .    .    NS0_prod
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*    *    **   *      Amino Acids
*    *    .    *      Nutrients
.    .    .    .      Vitamins
.    .    .    .      Trace Metals
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Figure 2A. HFN and NS0 Cell Growth by Screening Design Point

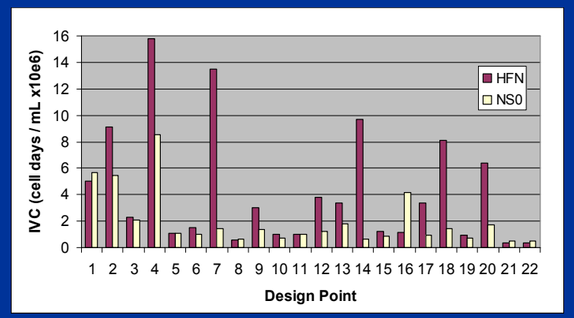
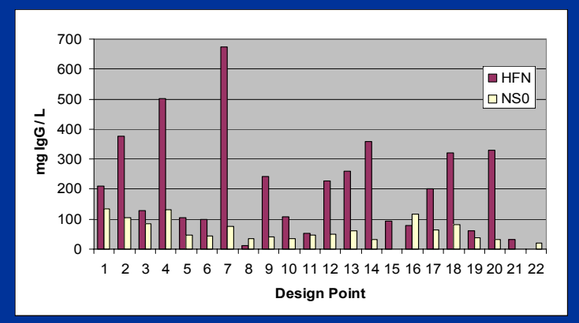


Figure 2B. HFN and NS0 Productivity by Screening Design Point



Case Study 2b: Optimization of Amino Acid and Nutrient Groups using a Surface Response Model (DoE) compared to a traditional Titration Method.

A partial cubic surface response model was used to design experiments for determining the optimal concentrations of amino acid and nutrient groups. Variable component groups were amino acid and nutrients groups, and all other groups were kept constant. For HFN cell growth, optima were 1.27x for the amino acid group and 0.37x for the nutrient group (Figure 3A). For HFN cell productivity, optima were outside the experimental range and another round of optimization will be necessary (Figure 3B). Traditional titration methods were used for comparison since previous screening experiments found no interactions between the groups (data not shown). However, there were differences between amino acid and nutrient group optima given by DoE and titration (Table3).

Figure 3A. Surface Response Plots for IVC (HFN)

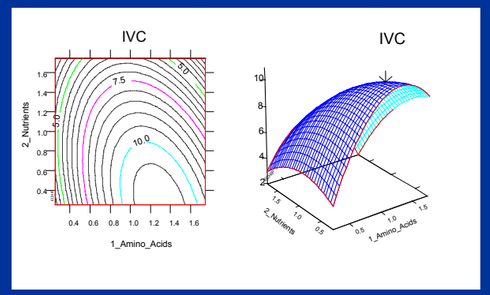


Figure 3B. Surface Response Plots for Productivity (HFN)

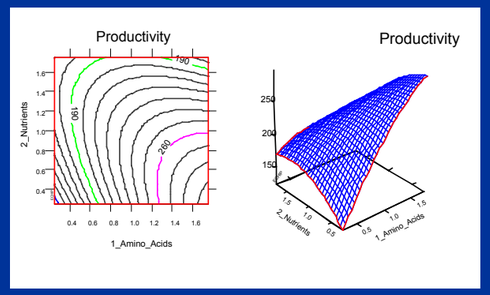


Table 3. Comparison of Design of Experiment Surface Response and Traditional Titration Method

	Optima by Method	
Group	DoE	Titration
Amino Acids	1.27x	1.54x
Nutrients	0.37x	1.02x

Conclusions

1. Combining different culture media design and optimization methods can result in rapid and efficient development of media.
2. Different cell lines have different nutrient requirements that can be detected using design of experiment screening methodologies.
3. Traditional (blending, component titration) and design of experiment methods (screening, surface response) can be used in a complementary way for efficient design and optimization of cell culture media.
4. We are examining the differences between amino acid and nutrient group optima given by DoE and titration to determine the best use of each method.

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