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 be accomplished using the most suitable methods, tools, and combinations of media contributing factors. In this work, we will show two of the many methods we employ for rational design of serum- and protein-free media for both hybridoma and myeloma cell lines. The first is a surface response model (DoE) based rational screening, followed by a traditional titration-based optimization. The second is a screening phase in which a layered factor design and optimization is performed. In this work we will focus on the optimization of IgG-producing hybridoma (HFN) and myeloma (NS0) cell lines. Two different types of cell lines were used for these studies. The hybridoma cell line, HFN, expresses an anti-fibronectin IgG and is a well-known model. The myeloma cell line, NS0, is a relatively new cell line and is being used in these studies to evaluate the need for additional component additions to the hybridoma cell line. The four variable component groups were: Amino Acids, Nutrients, Vitamins, and Trace Metals. A four-group factorial design and optimization were performed using ECHIP software version 6.0. The amino acid and nutrient groups had the greatest effect on both cell growth and productivity, while the vitamin and trace metal groups had the greatest effect on productivity.

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

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

Methods
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 be accomplished using the most suitable methods, tools, and combinations of media contributing factors. In this work, we will show two of the many methods we employ for rational design of serum- and protein-free media for both hybridoma and myeloma cell lines. The first is a surface response model (DoE) based rational screening, followed by a traditional titration-based optimization. The second is a screening phase in which a layered factor design and optimization is performed. In this work we will focus on the optimization of IgG-producing hybridoma (HFN) and myeloma (NS0) cell lines. Two different types of cell lines were used for these studies. The hybridoma cell line, HFN, expresses an anti-fibronectin IgG and is a well-known model. The myeloma cell line, NS0, is a relatively new cell line and is being used in these studies to evaluate the need for additional component additions to the hybridoma cell line. The four variable component groups were: Amino Acids, Nutrients, Vitamins, and Trace Metals. A four-group factorial design and optimization were performed using ECHIP software version 6.0. The amino acid and nutrient groups had the greatest effect on both cell growth and productivity, while the vitamin and trace metal groups had the greatest effect on productivity.

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

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

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 nutritional 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 complimentary 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.

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