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Technical Bulletin

Designing Feed Strategies for Fed-Batch CHO Cultures

Chinese Hamster Ovary (CHO) cell lines are widely used by the biopharmaceutical industry for production of therapeutic recombinant proteins and monoclonal antibodies. Optimizing bioreactor cell culture processes represents one of the most critical factors in determining the economic feasibility of a biopharmaceutical product. SAFC Biosciences™ has extensive experience developing feeding strategies and manufacturing concentrates, feeds and supplements. These components extend the length of CHO cultures, improve maximum cell densities and improve protein production and quality in fed-batch bioreactor cultures.

Developing a Feeding Strategy

A comprehensive process is required when developing a feeding strategy. A robust base medium must first be identified and proven in small- (spinners or shaker cultures) and large-scale (bioreactor) cultures. Spent medium analysis of the consumed amino acids, vitamins, carbohydrates and lipids and monitoring of the accumulating waste products (ammonia, lactate) during and after culture is a crucial step in designing supplements. SAFC Biosciences has the capability to assist with spent medium analysis through our Process Development department.

At SAFC Biosciences, our Cell Sciences and Development scientists use a variety of statistical and rational design methods to design and optimize feeds. Factorial matrix experiments are statistically designed experiments using Design of Experiment (DoE) software such as FusionPro® (S-Matrix Corp, CA). These experiments evaluate the performance of grouped media components such as amino acids, vitamins, iron, lipids and trace elements in cell culture at high and low concentrations. These experiments help to decipher the interactions of the individual groupings and hone-in on the best concentration of those components. Once the feeds have been developed, statistical methodology can also help to determine the optimum feeding schedules. Central composite designs can be utilized to determine the robustness of the feed(s) and analyze the relationships between feed components and cellular responses.

SAFC Biosciences imMEDIAte ADVANTAGE™ program can facilitate your experiments by providing small volumes of custom formulations for testing purposes. Our expertise allows us to manufacture concentrated feeds as one-part or multiple-part solutions. SAFC Biosciences also has a variety of feeds and concentrates for CHO cells which are chemically defined, animal-component free and do not contain glucose, L-glutamine, phenol red or hypoxanthine and thymidine, permitting their use with the dihydrofolate reductase ($DHFR^-$) gene amplification system.

Case Study: Development of a One-Part, Chemically Defined Bioreactor Feed for an IgG-Secreting CHO Cell Line

A study was undertaken to develop a chemically defined one-part feed and feeding schedule for an IgG-secreting CHO cell line in bioreactor cultures. The cell line was maintained in EX-CELL™ 302 Serum-Free Medium for CHO Cells (SAFC Biosciences Catalog No. 14324C) supplemented with L-glutamine and glucose.

Initial studies were performed in spinner flask cultures. Spent medium analysis was performed on un-fed cultures to determine the components that were being depleted and to develop a profile of metabolic waste production. These studies determined the appropriate levels of glucose and glutamine supplementation and led to the development of several different concentrated vitamin, amino acid and hydrolysate feeds. Soy hydrolysate was evaluated as it is an excellent source of amino acids and peptides in cell culture.

Statistical Design of Experiment (DoE) software designed a preliminary set of experiments to evaluate each feed individually and in various combinations. The results were analyzed by DoE software and a subset of feed combinations was designed and again tested in spinner flasks. The optimum feed combination (0.64:1 amino acid feed:vitamin feed) increased cell mass by 53% and increased IgG production by 65% in comparison to the control, when fed on alternating days beginning on Day 4 post inoculation. The soy feed either

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alone, or in combination with the amino acid and/or vitamin feed, did not produce significantly better results. Removing the soy hydrolysate allowed the development of a chemically defined feeding strategy.

The individual amino acid and vitamin feeds were reformulated, combined and manufactured by the imMEDIAte ADVANTAGE department into a 20X one-part feed. The formulation was named EX-CELL™ 66131 and was tested in bioreactor cultures. Based on spent medium analysis, EX-CELL 66131 was fed on alternating days beginning on Day 3 post inoculation. The bioreactor results were very similar to the spinner flask results; CHO cells fed EX-CELL™ 66131 in conjunction with D-(+)-Glucose and L-Glutamine yielded IgG titers 68% higher than control bioreactors fed only Glucose and L-Glutamine, and 113% higher IgG titers to control bioreactors which were unfed. Furthermore, EX-CELL 66131 did not increase the metabolic waste profile in comparison to the glucose/glutamine fed control.

This case study indicates that it is possible to use a combination of process development tools including spent medium analysis and statistical software to design, rapidly evaluate and optimize a feeding strategy which can be utilized in small scale cultures and is scalable to larger bioreactor cultures.

For further information regarding this study, or for help designing a medium or feed, please visit our website at www.safcbiosciences.com or by contact our Technical Services department at technicalservices@sial.com.

Figure 1

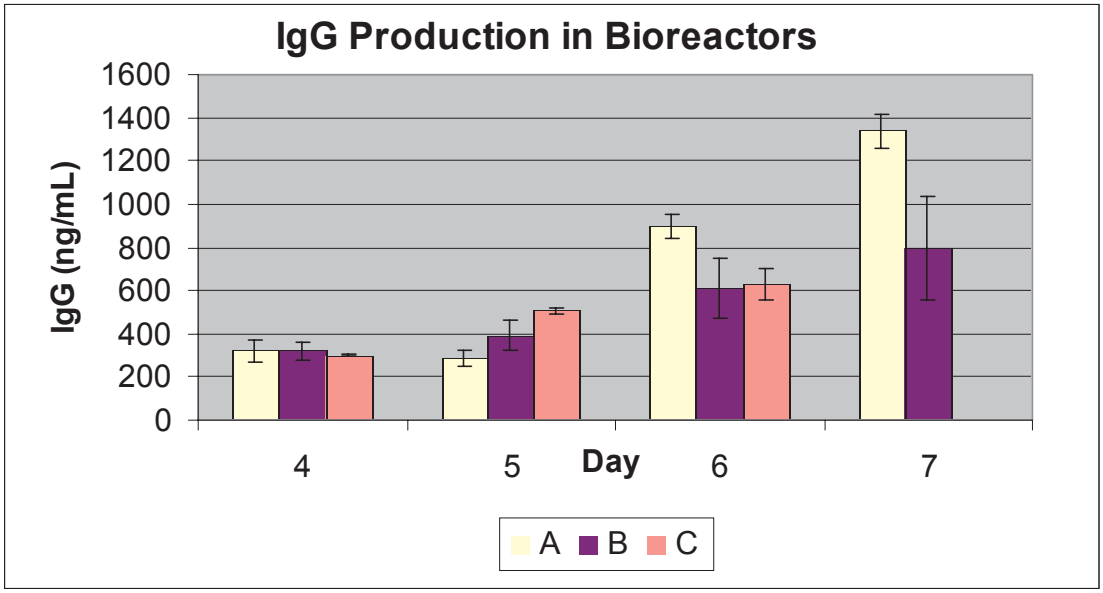

Figure 1: IgG Production by B13-24 Cells in EX-CELL 302. Bioreactor A was supplemented with EX-CELL 66131 20X one-part feed, L-Glutamine and Glucose. Bioreactor B was supplemented with only L-Glutamine and Glucose; Bioreactor C was un-fed.

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