

## Research Report

# Development and Application of a Chemically Defined Bioreactor Feed for CHO Cells

**Jody Beckmann, Benito Delgado, Mason Williams, Sandy McNorton, Dennis Cooley, Jeanette Hartshorn**  
Cell Sciences & Development, SAFC, Saint Louis, Missouri 63103 and Lenexa, Kansas 66215

### Abstract

To increase the production of recombinant IgG in a Chinese Hamster Ovary (CHO) cell line in bioreactor cultures, statistical experimental design methods were employed to develop a chemically defined, one-part nutrient feed referred to as EX-CELL<sup>®</sup> 66131. 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. These results illustrate a successful methodology for the development of an effective bioreactor feed formulation for a recombinant CHO cell line.

### Introduction

Improvement of bioreactor processes for large-scale production of recombinant protein therapeutics is an area of significant interest for many industrial biotechnology companies. Large-scale CHO-based manufacturing is complex and has many facets including cell line development, media and feed optimization, bioreactor process design and downstream protein retrieval and purification. Batch and fed-batch modes of culture are frequently employed as they offer ease of operation, scalability, good process control and concentrated end-product.

In addition to manipulating process parameters to control temperature, pH and gas exchange, the balanced feeding of carbohydrates (Altamirano et al), amino acids (deZengotita et al), vitamins (Blaker and Pirt), lipids, hydrolysates, iron carriers and growth factors at strategic intervals can help maintain the nutritional environment of cells during the course of the fed-batch culture. This can increase maximum viable cell density, extend culture duration, increase in protein production, control and improve glycosylation properties (Chen and Harcum, Kuwae et al, Wong et al, Zanghi et al) and limit the production of metabolic byproducts.

Medium mixing and statistical experimental design methods for media and feed design and process optimization are becoming more prevalent (Gifford et al, Sandadi et al, Castro et al). Statistical methodology allows concurrent screening of

several formulations and media combinations by performing mixing experiments, which can lead to faster and more efficient media and feed development. Factorial design and response surface methods are also used to determine optimal bioreactor parameters and their acceptable ranges.

The goal of this study was to utilize statistical experimental design methods to develop a one-part chemically defined feed for an IgG-secreting CHO cell line, which would be scaleable for use in bioreactors. Preliminary experiments were performed using B13-24 CHO cells in commercially available EX-CELL 302 Serum-Free Medium for CHO Cells. Unfed spinner and bioreactor cultures were initially used to evaluate cell growth, nutrient consumption and IgG production. Spent medium analysis is an integral part of feed process development and determines cell-specific requirements. Analytical data obtained from spent medium analysis indicated that specific amino acids and vitamins were being consumed. FusionPro<sup>™</sup> DoE (Design of Experiments) statistical software (version 7.3.20, S-Matrix Corp., Eureka, CA) was used to generate a set of 20 experimental conditions (performed in spinners) to evaluate different combinations of concentrated vitamin, amino acid and soy hydrolysate feeds. The formulations of the concentrated amino acid, vitamin and hydrolysate feeds were designed an individual solutions based on the formulation of EX-CELL 302 medium and the consumption of specific components.

Statistical analysis of data from the spinner flask experiments was used to rank the feed combinations which produced significant effects and predict a subset of optimized feed concentrations which would further increase productivity. These feeds were evaluated in a second phase of spinner flask studies to finalize the optimum feed concentrations. At this point, each feed was manufactured individually. The highest cell mass was achieved using a feed combination comprised of 64% of the amino acid group with 100% of the vitamin group. The same feed yielded a titer increase of 65%. The soy hydrolysate feed had negligible positive impact on cell growth and productivity. Based on the results of these experiments, the optimal feeding strategy was determined to be the combination of a 64% amino

acid feed with a 100% vitamin feed. This formulation was then manufactured by SAFC imMEDIATE ADVANTAGE™ (IA) department as a one-part, 20X concentrated feed known as EX-CELL 66131 (SAFC Product # 66131C). Bioreactor cultures fed with EX-CELL 66131 had final average IgG productivity titers 68% higher than the Gluc/L-Gln Fed Control and 113% higher than the Non-Fed Control.

## Materials

### Cell Line

- B13-24, IgG<sup>4</sup> producing CHO Cell Line, American Type Culture Collection, Catalog No. CRL-11397

### Media and Reagents

- EX-CELL 302 Serum-Free Medium for CHO Cells, SAFC, Catalog No. 14324C
- EX-CELL 66131, SAFC, Product No. 66131C
- L-Glutamine, 200 mM solution, Sigma-Aldrich, Catalog No. G7513
- D-(+)-Glucose solution, Sigma-Aldrich, Catalog No. G8769
- Bioreactor pH Adjustment Solution, Sigma-Aldrich, Catalog No. B1185

### ELISA Kit

- Human IgG ELISA Kit, Zeptometrix, Catalog No. 0801182

## Cell Culture Methods

### Spinner Flask Experiments

Initial experiments were performed using EX-CELL 302 medium (supplemented with 4 mM L-glutamine) in 500 mL Bellco spinners with 250 mL working volume. B13-24 CHO cells were seeded at  $0.4 \times 10^6$  viable cells/mL and maintained at 120 rpm, 37 C and 7.5% CO<sub>2</sub>. Glucose was maintained between 1 - 5 g/L, and L-glutamine was maintained between 1 - 4 mM. Amino acid, vitamin and/or hydrolysate feeds were supplemented on days 4, 6 and 8. Analysis of daily samples included determinations of cell viability, density, metabolic trending and osmolality. Cells were counted using a Cedex Automated Cell Analyzer (Innovatis AG, Germany). Glucose, lactate, glutamine, ammonium and glutamate concentrations were determined using a BioProfile 400 Analyzer, (Nova Biomedical, Waltham, MA). Osmolality was determined using a Vapro® Vapor Pressure Osmometer (Wescor Inc., Logan, UT) Additional samples were centrifuged to remove cell debris and the supernatants frozen at -20 C. Productivity analysis was performed using a human IgG ELISA kit, following the manufacturer's protocol.

**Table 1**

Test Condition	Amino Acids (%)	Soy Hydrolysate (%)	Vitamins (%)
1	64	0	100
2	55	100	58
3	44	100	73
4	56	0	73
Control	0	0	0

## Bioreactor Experiments

Biostat B 5L bioreactors (B. Braun Biotech International) were seeded at  $0.2 \times 10^6$  viable cells/mL in 4.5 L initial working volumes of EX-CELL 302 culture medium supplemented with 4 mM L-Glutamine. Parameter set points were as follows and controlled using MFCS/win control software (version 2.1, B Braun Biotech International): DO 50%; pH 6.9 - 7.3 (controlled with CO<sub>2</sub> and Bioreactor pH Adjustment Solution); temperature 37 C; agitation 100 rpm. Analysis of daily samples included determinations of cell viability, density, metabolic trending and osmolality. Samples were frozen for later productivity analysis via ELISA. Experimental conditions were set up as described in Table 2.

**Table 2**

Experimental Condition	Feed Details
Non-Fed Control (n=2)	No Feeding
Glucose/L-Glutamine Fed Control (n=3)	Fed Glucose up to 4 g/L when at 2 g/L or below Fed L-Glutamine up to 4 mM when at 2 mM or below
EX-CELL 66131 (n=2)	Fed Glucose up to 4 g/L when at 2 g/L or below Fed L-Glutamine up to 4 mM when at 2 mM or below Fed 50 mL of EX-CELL 66131 on Day 3 and Day 5 of culture

## Results

### Spinner Flask Experiments

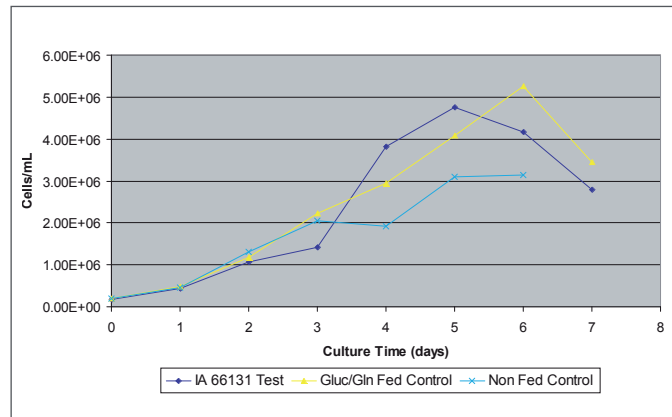
Analytical data obtained from spent medium analysis indicated that specific amino acids (specifically asparagine and cystine) and vitamins (biotin and cyanocobalamin) were being consumed.

The contour plot (data not shown) indicates by color that the expected concentration ranges of component groups in the feed necessary to achieve maximum productivity titers are 25 - 92% of the starting amino acid concentration and >54% of the starting vitamin concentration. Results from the predicted optimized conditions (Table 2), show that the feeds yielded a 20 - 53% increase in total viable cell mass and a 21 - 73% increase in productivity titer when compared to the Glucose/L-Glutamine fed control. The highest cell mass was achieved using a feed combination comprised of an additional 64% of the amino acid group with 100% of the vitamin group (data not shown).

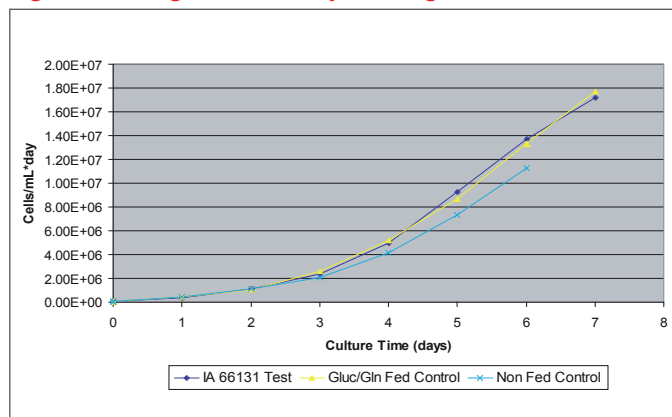
Two feed combinations yielded similar increases in productivity titers, one of which contained soy hydrolysate and the other one no hydrolysate. A feed comprised of a combination of an additional 64% of the amino acid group with 100% of the vitamin group yielded a titer increase of 65%, while a feed comprised of an additional 44% of the amino group, 100% of the soy hydrolysate group and 70% of the vitamin group yielded an increase of 73% (data not shown). The soy hydrolysate had negligible positive impact on cell growth and productivity. Based on the results of these experiments, the optimal feed formulation was determined to be the combination of an additional 64% of the amino acid group with 100% of the vitamin group. This formulation was then manufactured as IA 66131.

The results from these experiments indicate that CHO cells in the Non-Fed Control only grew to a maximum cell density of approximately  $3.0 \times 10^6$  and dropped to 50% viability by Day 6 of culture (which was one day earlier than the other two fed conditions). The Glucose/L-Glutamine Fed Control and the EX-CELL 66131 bioreactors reached comparable viable cell densities, both peaking at approximately  $5.0 \times 10^6$  viable cells/mL (Figure 1a). While the Glucose/L-Glutamine Fed Control bioreactors peaked on Day 6 and the EX-CELL 66131 bioreactors peaked on Day 5, these two test conditions had equivalent cumulative growth curves (Figure 1b). Additionally, these two conditions showed similar reductions in viabilities with both conditions falling to less than 50% viable cells in culture by Day 7. These results suggest that feeding EX-CELL 66131 in combination with Glucose and L-Glutamine does not affect growth or viability when compared to cultures fed only Glucose and L-Glutamine (Figure 1c).

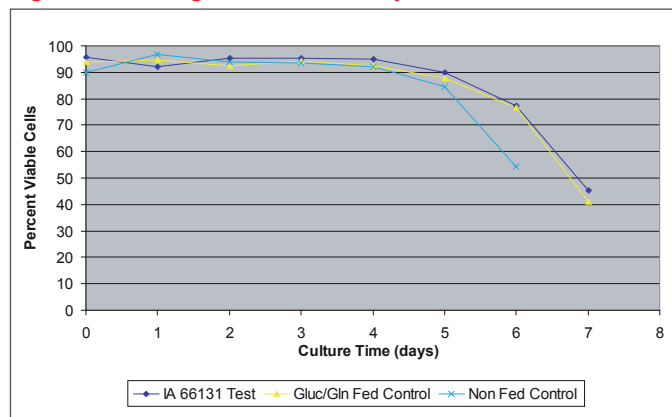
**Figure 1a. Average Viable Cell Density**



**Figure 1b. Integrated Cell Days Average**

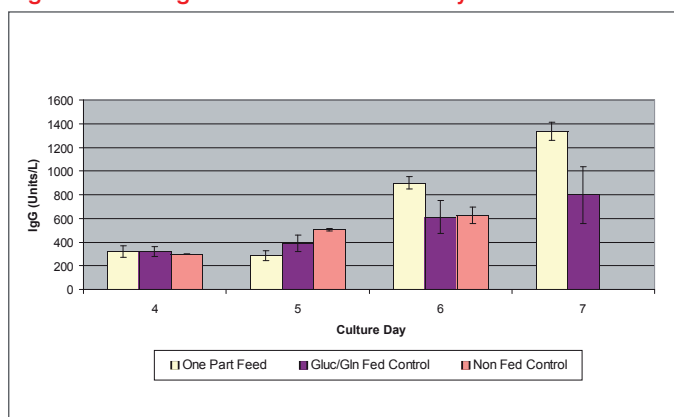


**Figure 1c. Average Percent Viability**



Productivity titers achieved in bioreactors were comparable to those achieved at small scale (Figure 2). In bioreactors, cultures fed with CHO Feed EX-CELL 66131 had final average IgG productivity titers 68% higher than the Glucose/L-Glutamine Fed Control and 113% higher than the Non-Fed Control. These results indicate that the improvements in productivity titers achieved by using EX-CELL 66131 with a CHO cell line are scaleable to bench-scale bioreactors.

**Figure 2. Average Bioreactor Productivity**



### Metabolic Trending

Figure 3a, Glucose and L-Glutamine Analysis. Both the Glucose/L-Glutamine Fed Controls and the IA 66131 Feed conditions were fed Glucose and/or L-Glutamine on Days 3, 4, 5 and 6 of culture. For both conditions, Glucose was successfully maintained between 1.5 g/L and 3.5 g/L, and L-Glutamine was successfully maintained between 1 mM and 6 mM. The Non-Fed Control depleted almost all Glucose and L-Glutamine by Day 4 of culture.

Figure 3b, Waste Production Analysis. The Glucose/L-Glutamine Control and the IA 66131 Test condition showed very similar waste production trends. Since addition of Glucose and L-Glutamine to cell cultures usually increases the amount of Ammonia, Lactate and Glutamate produced, it was not surprising that the Non-Fed condition produced the least metabolic waste.

### Osmolality

The highest osmolality reached for the IA 66131 Test Condition was 420 mOsm/kg, the highest reached for the Glucose/L-Glutamine Control was 400 mOsm/kg and the highest reached for the Non-Fed Control was 357 mOsm/kg. Osmolality was not markedly different between the Glucose/L-Glutamine Fed Control and the IA 66131 Test condition. (Data not shown.)

**Figure 3a.**

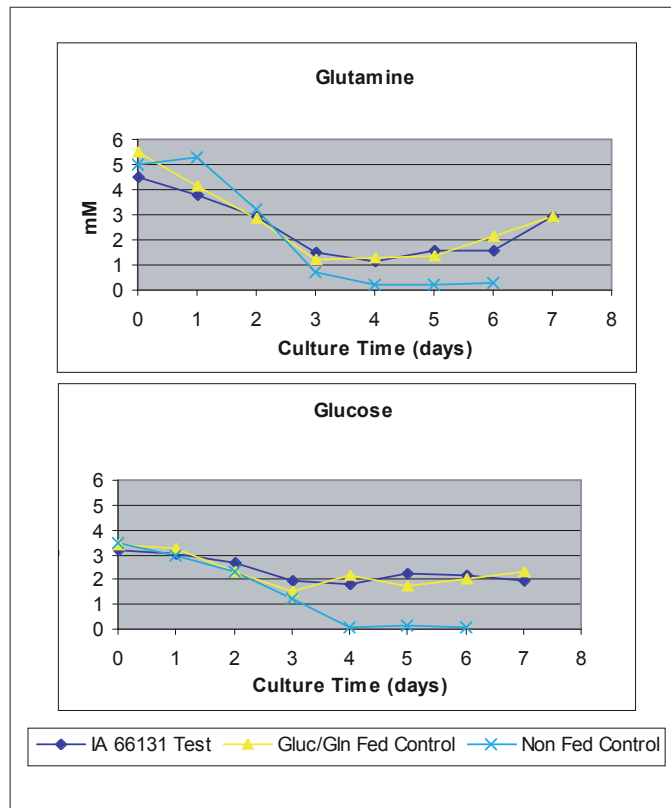
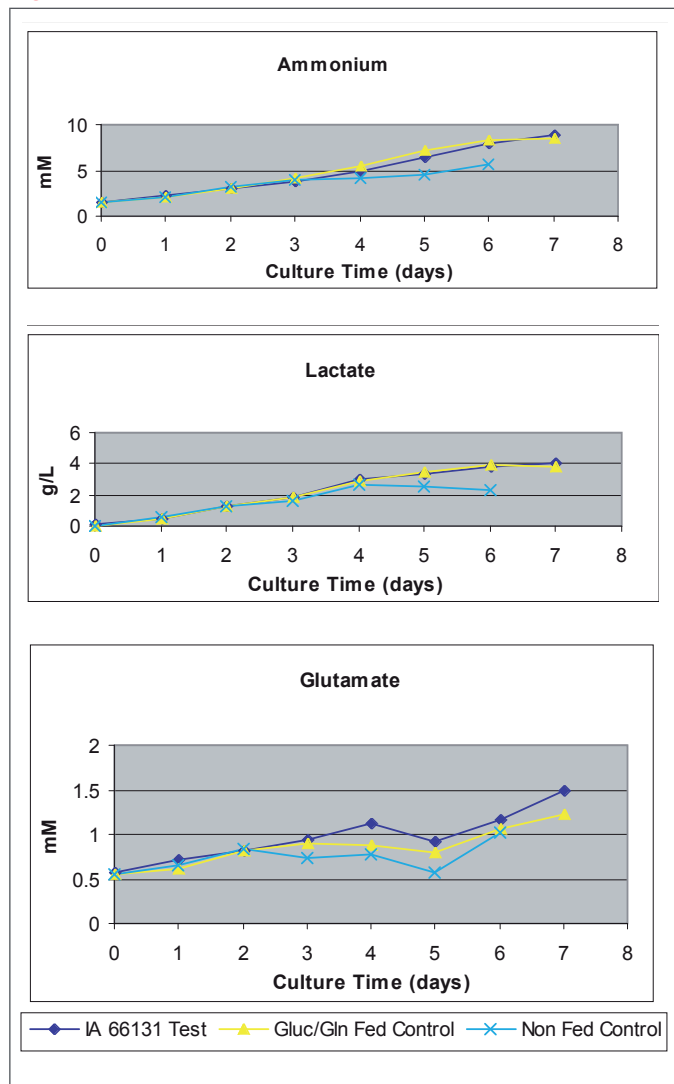


Figure 3b.



## Conclusions

- A successful methodology for the development of an effective bioreactor feed formulation for a recombinant CHO cell line has been demonstrated.
- Development data for chemically defined feed IA 66131 obtained at the small-scale spinner flask level was consistent with data obtained at the 5 L bench bioreactor scale.

For more information, visit [safcglobal.com](http://safcglobal.com) or contact SAFC

USA: + 1-913-469-5580 • [info-na@sial.com](mailto:info-na@sial.com)

Europe: +44 (0)1264-333311 • [info-eu@sial.com](mailto:info-eu@sial.com)

Asia/Pacific: +61 (0)2 9841 0555 • [auscustserv@sial.com](mailto:auscustserv@sial.com)

- At small scale, cultures fed with IA 66131 in conjunction with Glucose and L-Glutamine yielded an average of 65% higher IgG titers than spinners fed with only Glucose and L-Glutamine. At the 5 L bioreactor scale, cultures fed with IA 66131 using the same feeding scheme yielded an average of 68% higher IgG titers. Bioreactor cultures fed with IA 66131 yielded an average of 113% higher IgG than non-fed control bioreactors.
- When added to bioreactor cultures, IA 66131 did not significantly increase waste production or osmolality.

## References

1. Altamirano C., Paredes C., Illanes A., Cairo J.J., Godia F., Strategies for fed-batch cultivation of t-PA producing CHO cells: substitution of glucose and glutamine and rational design of culture medium. (2004) *Journal of Biotechnology* 110:171-179.
2. Blaker G.J., Pirt S.J., The uptake of vitamins by mouse fibroblast cells (strain LS) during growth in a chemically defined medium. *Journal of Cell Sciences* (1971) 8:701-708.
3. Castro P.M.L., Hayter P.M., Ison A.P., Bull A.T., Application of a statistical design to the optimization of culture medium for recombinant interferon-gamma production by Chinese hamster ovary cells. *Appl Microbiol Biotechnol* (1992) 38:84-90.
4. Chen P., Harcum S.W., Effects of amino acid additions on ammonium stressed CHO cells. *Journal of Biotechnology* (2005) 117(3):277-286.
5. deZengotita V.M., Abston L.R., Schmelzer A.E., Shaw S., Miller W.M., Selected amino acids protect hybridoma and CHO cells from elevated carbon dioxide and osmolality. (2002) *Biotechnology and Bioengineering* 78(7):741-752.
6. Gifford J., Albee A., Deeds Z.W., Delong B., Kao K., Ross J.S., Caple M.V., An efficient approach to cell culture medium optimization - a statistical method to medium mixing. *Sigma-Aldrich Biotechnology*.
7. Kuwae S., Ohda T., Tamashima H., Miki H., Kobayashi K. Development of a fed-batch culture process for enhanced production of recombinant human antithrombin by Chinese hamster ovary cells. *Journal of Bioscience and Bioengineering* (2005) 100(5):502-510.
8. Sandadi S., Ensari S., Kearns B., Heuristic optimization of antibody production by Chinese hamster ovary cells. *Biotechnology Progress* (2005) 21:1537-1542.
9. Sandadi S., Ensari S., Kearns B., Application of fractional factorial designs to screen active factors for antibody production by Chinese hamster ovary cells. *Biotechnology Progress* (2006) 22:595-600.
10. Wong D.C.F., Wong K.T.K., Goh L.T., Heng C.K., Yap M.G.S., Impact of dynamic online fed-batch strategies on metabolism, productivity and N-glycosylation quality in CHO cell cultures. *Biotechnology and Bioengineering* (2005) 89(2):164-177.
11. Zanghi J.A., Schmelzer A.E., Mendoza T.P., Knop R.H., Miller W.M., Bicarbonate concentration and osmolality are key determinants in the inhibition of CHO cell polysialylation under elevated pCO<sub>2</sub> or pH. *Biotechnology and Bioengineering* (1999) 65(2):182-191.

### Warranty, Limitation of Remedies

SAFC warrants to the purchaser for a period of one year from date of delivery that this product conforms to its specifications. Other terms and conditions of this warranty are contained in SAFC written warranty, a copy of which is available upon request. ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING THE IMPLIED WARRANTY OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE, ARE EXCLUDED. In no case will SAFC be liable for any special, incidental, or consequential damages arising out of this product or the use of this product by the customer or any third party based upon breach of warranty, breach of contract, negligence, strict tort, or any other legal theory. SAFC expressly disclaims any warranty against claims by any third party by way of infringement or the like. THIS PRODUCT IS INTENDED FOR PURPOSES DESCRIBED ONLY AND IS NOT INTENDED FOR ANY HUMAN OR THERAPEUTIC USE.

Additional Terms and Conditions are contained in the product Catalog, a copy of which is available upon request.

©2012 Sigma-Aldrich Co. LLC. All rights reserved. SAFC and SIGMA-ALDRICH are trademarks of Sigma-Aldrich Co. LLC, registered in the US and other countries. EX-CELL and imMEDIATE ADVANTAGE are trademarks of Sigma-Aldrich Co. LLC, registered in the US and other countries. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the SAFC website at [www.safcglobal.com](http://www.safcglobal.com) and/or on the reverse side of the invoice or packing slip.

July 2012 2008 R032