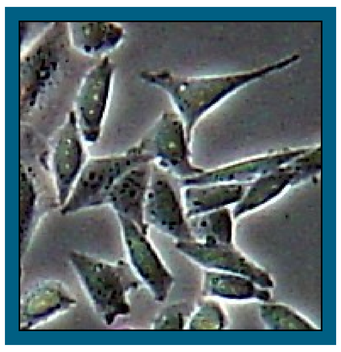

Development of chemically defined media for extended growth and enhanced productivity in CHO-K1 and CHO DG44 cultures

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Introduction

Chinese Hamster Ovary (CHO) cells are widely used in the biopharmaceutical industry for the production of recombinant proteins. The current strict regulatory norms for biopharmaceutical industry has led to the wide use of serum-free culture processes including chemically defined (CD) media. A significant amount of time and investment is required on the part of a business to develop an optimal CD media, which fits the needs of both the process and the company. Many companies may not have the resources to develop their own CD media. In response to customer requirements, Kerry has developed the Kerry CHO medium for extended growth and enhanced recombinant protein production. The Sheff-CHO CD complete media has been optimized in terms of Osmolality so that it can be seamlessly used with Kerry's individual plant-based hydrolysates as well as with our series of Sheff-CHO systems.

Sheff-CHO CD complete is a powdered, animal component free, protein free, chemically defined media that has demonstrated compatibility with multiple CHO cell lines and substantially improves cell growth/viability as well as enhanced product titers. The developmental formulation of the Sheff-CHO CD complete medium was screened in two different IgG expressing CHO DG44 dhfr- cell lines and one CHO-K1 expressing recombinant secreted embryonic alkaline phosphatase (SEAP). The viable cell density, viability, nutrient profiles and product titers obtained with Sheff-CHO CD complete medium were compared to commercially available CD medium for CHO cells in multiple experiments in shake flasks**.** To analyze the scalability of the Sheff-CHO CD complete medium, multiple rounds of fed-batch bioreactor experiments were performed in the various CHO models. The supernatants were collected for assessing the effect of the media on the production of IgG or SEAP. To test the efficacy of the Sheff-CHO CD complete medium with plant hydrolysates, a variety of Kerry's supplements were also tested with the Sheff-CHO CD complete medium.

Materials and Methods

CHO-K1: Data was collected using a transfected CHO-K1 line engineered to express SEAP and adapted to serum-free suspension culture. For bioreactor experiments, cultures were seeded at 0.8 x10^6 cells/mL (cell line C) and maintained at 37°C throughout the experiment. 1L Dasgip bioreactors were operated at a 500ml working volume, 6.8-7.2 pH range controlled via Na_2CO_3 and CO_2 addition, 50% DO with a 0.5-1.5 mL/min sparged gas flow rate and 150rpm agitation rate. For cultures grown in the commercially available chemically defined, media, the CHO specific basal media was supplemented with 0.1% Pluronic F-68, 5 mM L-glutamine, 0.75 mg/mL G-418 and 10 mg/L Sheffield rInsulin ACF. For the cells grown in Sheff-CHO CD complete media, this basal medium was supplemented only with 0.75 mg/ml G-418 and 10 mg/L Sheffield rInsulin ACF.

CHO DG44: Data was collected using a transfected CHO DG44 DHFR- line engineered to express IgG and adapted to serum-free suspension culture. For bioreactor experiments, cultures were seeded at either 1.0 x 10^6 cells/mL (cell line A) or 0.8 x 10^6 cells/mL (cell line B) and maintained at 37 °C from days 0-3, then shifted to 33 °C for the remainder of the experiment. 1L Dasgip bioreactors were operated at a 500ml working volume, 6.8-7.2 pH range controlled via Na_2CO_3 and CO_2 addition, 50% DO with a 0.5-1.5 mL/min sparged gas flow rate and 150 rpm agitation rate. For cultures grown in the commercially available chemically defined media, the CHO specific basal media was supplemented with 0.1 % Pluronic F-68, 5 mM L-glutamine, 10 mg/L Sheffield rInsulin ACF and 25 nM methotrexate. For the cells grown in Sheff-CHO CD complete media, this basal medium was supplemented only with 10 mg/L Sheffield rInsulin ACF and 25nM methotrexate.

Supplement/Sample Preparation: A 100 g/L stock of the supplements was prepared in the respective basal medium was and used for achieving the supplementation of the corresponding conditions. Supernatant samples were collected on day 6, 8, 10, 12, 13 and 14 and analyzed by HPLC for either SEAP or IgG concentration. The cultures were monitored on each day by removing culture samples and analyzing on the Nova Biomedical Bioprofile Flex bioanalyzer, for cell density/viability data along with nutrient and metabolite profiles (not shown).

Summary

The use of Sheff-CHO CD complete medium resulted in an enhanced performance in terms of cell density/viability and recombinant protein productivity for both CHO DG44 and CHO-K1 cell lines. As observed in Figures 1 and 2, the Sheff-CHO CD complete media greatly extends the viability of the cell line A and also increases the IgG titer almost two-folds on days 10 and 12. In Figure 3, the cell line B cultured in Sheff-CHO CD complete media shows an extended viability as compared to the commercial CD media control. The IgG titer of cell line B cultured in Sheff-CHO CD complete media was twice that of the commercial CD media control, as seen in Figure 4. For the CHO-K1 cells, the Sheff-CHO CD complete media resulted in a higher peak viable cell density as compared to the commercial CD media control, represented in Figure 5. As see in Figure 6, the SEAP yield in commercial CD media and in the Sheff-CHO CD complete media is very similar . The compatibility of the Sheff-CHO CD complete medium with the Kerry's complex supplements is demonstrated in Figures 7 and 8. The supplementation enhances the titers of cultures not only in the commercial CD media but also in the Sheff-CHO CD complete media. These results along with the availability of the Sheff-CHO CD complete medium as a dry powder medium (DPM) demonstrate that the Sheff-CHO CD complete medium is a superior option as a complete chemically defined medium for a variety of CHO cells lines.

Figure 1: CHO DG44 cell line A

Viable cell density (VCD) and Cell viability (CV)

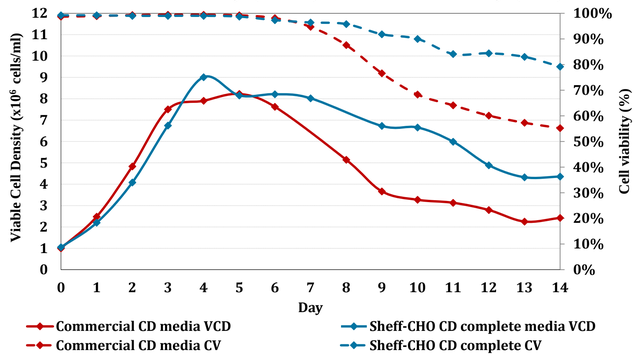
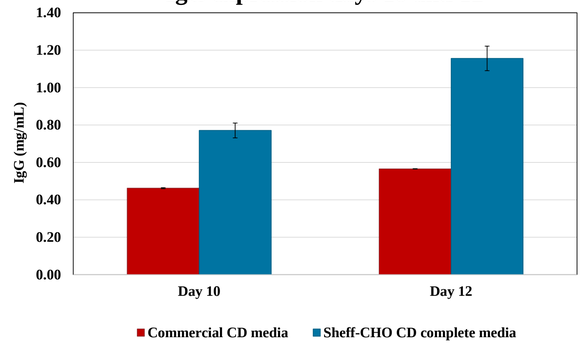


Figure 2: CHO DG44 cell line A

IgG expression days 10 and 12



Figures 1 and 2: Bioreactor cultures of CHO DG44 cell line A were cultured in commercial CD media and Sheff-CHO CD complete media. The effects of the different media on viable cell density, culture viability and IgG titer was assessed to determine overall culture health and productivity. The Sheff-CHO CD complete media demonstrated the ability to more than double the IgG yield as compared to the commercially available CD media. The observed growth patterns were better for the Sheff-CHO CD complete media.

Figure 3: CHO DG44 cell line B

Viable cell density (VCD) and Cell viability (CV)

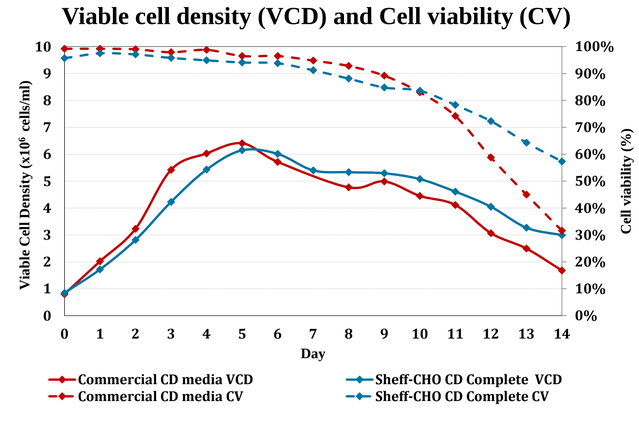
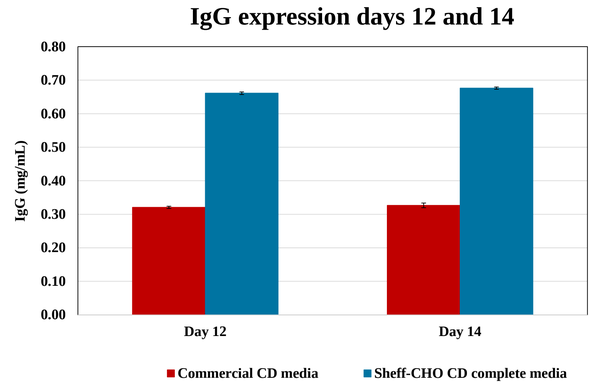


Figure 4: CHO DG44 cell line B

IgG expression days 12 and 14



Figures 3 and 4: Bioreactor cultures of CHO DG44 cell line B were cultured in commercial CD media and Sheff-CHO CD complete media. The effects of the different media on viable cell density, culture viability and IgG titer was assessed to determine overall culture health and productivity. The Sheff-CHO CD complete media demonstrated the ability to more than double the IgG yield as compared to the commercially available CD media. The observed growth patterns were better for the Sheff-CHO CD complete media.

Figure 5: CHO-K1 cell line C

Viable cell density (VCD) and Cell viability (CV)

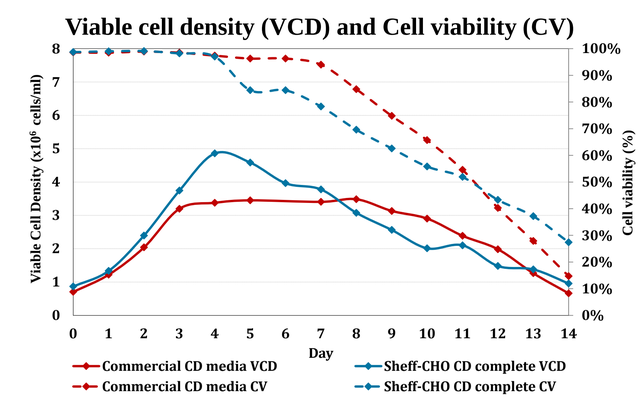
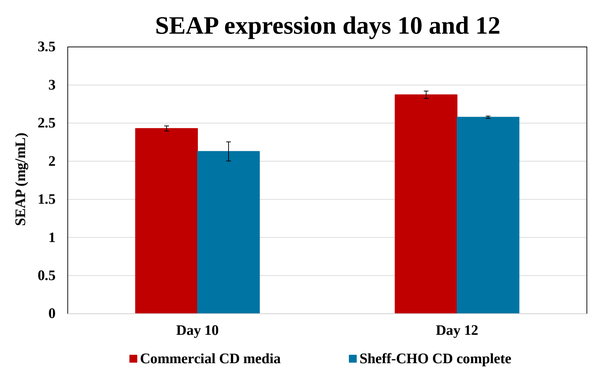


Figure 6: CHO-K1 cell line C

SEAP expression days 10 and 12



Figures 5 and 6: Bioreactor cultures of CHO-K1 cell line C were cultured in commercial CD media and Sheff-CHO CD complete media. The effects of the different media on viable cell density, culture viability and IgG titer was assessed to determine overall culture health and productivity. The Sheff-CHO CD complete media demonstrated the ability to increase the peak viable cell density and have similar SEAP yield as compared to the commercially available CD media. The observed growth patterns were better for the Sheff-CHO CD complete media.

Figure 7: CHO DG44 cell line A

Viable cell density (VCD) and Cell viability (CV)

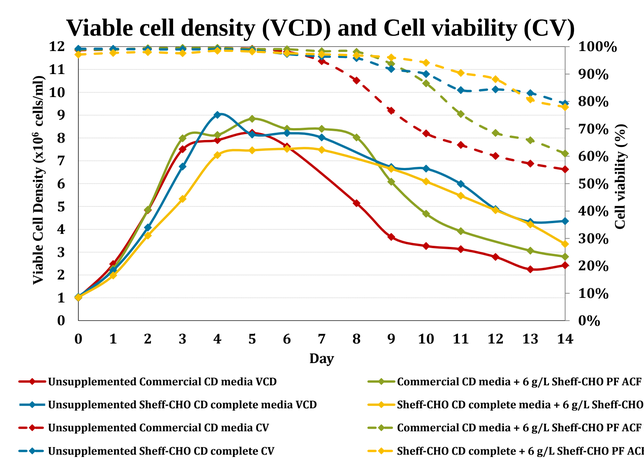
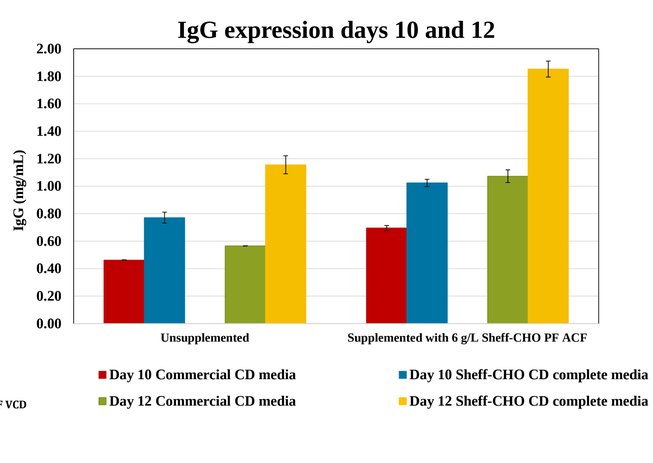


Figure 8: CHO DG44 cell line A

IgG expression days 10 and 12



Figures 7 and 8: Bioreactor cultures of CHO DG44 cell line A were cultured in commercial CD media and Sheff-CHO CD complete media. To demonstrate the compatibility of the Sheff-CHO CD complete media with Kerry's complex supplements, the media was supplemented with 6 g/L Sheff-CHO PF ACF. The supplementation resulted in a significant increase in the IgG titer as compared to the unsupplemented media control in both the Sheff-CHO CD complete media as well as the commercial CD media.