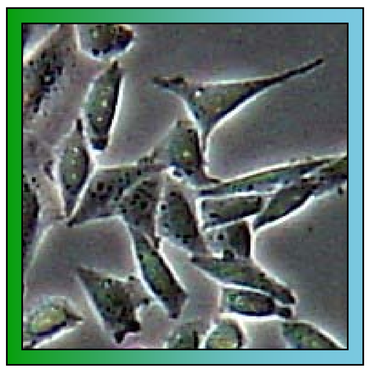

Use of chemically defined supplement systems Sheff-Pulse CD and Sheff-CHO CD for the improvement of cell growth and recombinant protein titer.

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Introduction

There has been a growing trend towards the use of Chemically defined media and supplements in the biopharmaceutical industry in an effort to improve product consistency. To meet this trend Kerry has developed CHO specific CD supplements with the intent to increase biopharmaceutical process yield, improve culture health and reduce media-related cost. Sheff-CHO CD v1 or Sheff-CHO CD v2 can be used as a single supplement to improve product titer in CHO-K1 and CHO-DG44 cells respectively. Sheff-Pulse CD v1 is a chemically defined feed supplement designed to improve product titer in CHO cells.

Materials and Methods

A CHO-K1 cell line modified to produce secreted alkaline-phosphatase (SEAP) and CHO-DG44 dhfr- antibody producing cell line was used to develop the CD supplements. Seventy prototype supplements were screened in both CHO models. The screening protocol utilized multiple rounds of shake flask experiments and were performed in commercially available chemically defined (CD) media. Sheff-Pulse CD performance was analyzed both shake flasks bioreactors.

CHO-K1: *Data was collected using a transfected CHO-K1 line engineered to express SEAP and adapted to serum-free suspension culture. Cultures were grown in 125 ml shake-flasks with an initial working volume of 35 ml. Triplicate cultures were seeded at 4.0 x10^5 cells/ml and incubated at 37°C in 5% CO2 at 130 rpm for 14 days. All basal media were supplemented with 0.2% pluronic, 4mM L-glutamine and 0.75 mg/ml G-418.*

CHO DG44: *Data was collected using a transfected CHO DG44 DHFR- line engineered to express IgG and adapted to serum-free suspension culture. Cultures were grown in 125 ml shake-flasks with an initial working volume of 35 ml. Triplicate cultures were seeded at either 6.0 or 1.0 x10^5 cells/ml and incubated at 37°C from days 0-3 and 33°C from day 3 onwards. Experiments were maintained for up to 14 days at in a 5% CO2 environment with an agitation rate of 130 rpm.*

Supplement/Sample Preparation: *Two of the prototype supplements were named Sheff-CHO CD v1 and Sheff-CHO CD v2. These supplements are prepared by hydrating with cell culture grade water to a 100x concentration and then filter sterilized. It was applied to both CHO-K1 and CHO-DG44 clones at 0.5, 1.0 and 2.0x concentration respectively on day four of culture. Sheff-Pulse CD v1 was diluted in cell culture grade water to a 100x concentration.*

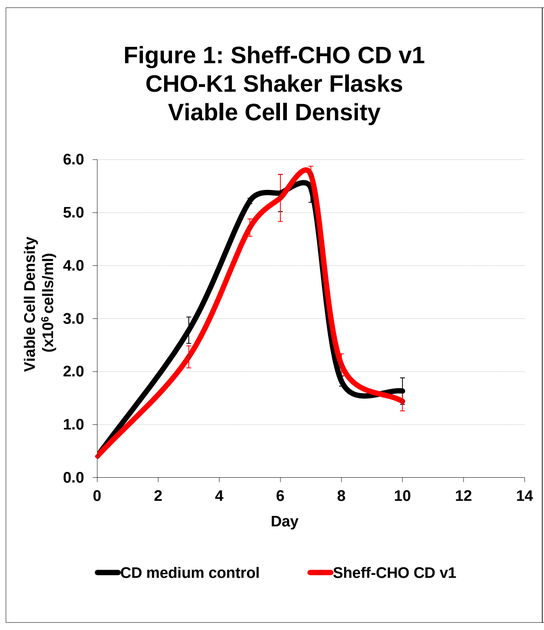
Bioreactor experiments: *Sheff-Pulse CD1 was applied to the CHO DG44 line at a 3.0x concentration on days 3, 5, 7, 9, and 12. Competitor feed supplements were used at the recommended dosages and times per manufacturer's instructions. Day 10, 12 supernatant samples were analyzed by HPLC for SEAP or IgG concentration. On multiple days, culture samples were removed and analyzed using the Nova Flex bioanalyzer yielding cell density/viability data in addition to nutrient and metabolite profiles.*

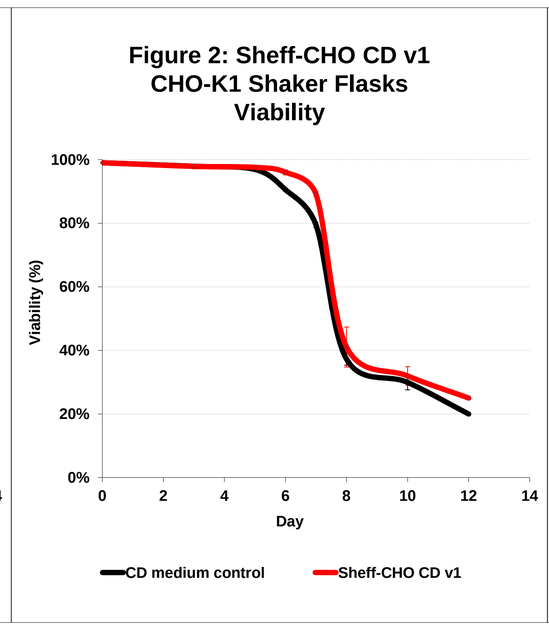
Summary

Three prototype CD supplements were developed for use in CHO cell lines. Sheff-CHO CD v1 improved titer by 40% when supplemented into a commercially available CD media at 1.0x concentration in our CHO-K1 clone (Figure 3). Sheff-CHO CD v2 improved cell viability and increased product titer by >100% when applied to CHO-DG44 dhfr IgG producing cell line (Figure 3 and 4). Interestingly, the dosage concentration was very important with the CHO-DG44 clone with the lesser concentration giving the best results.

Sheff-Pulse CD v1 was identified from shake flask experiments (data not shown) and it's efficacy was reconfirmed in bioreactors where it's performance was compared to a CD media and a competitor CD feed. This feed supplement did not increase cell density but significantly increased cell viability (Figure 7). Viability dropped from 90% to 0% with the control and competitor feed from day 5 to day 10. Supplementation with Sheff-Pulse CD v1 kept cell viability in the 70-79% range through to the 15th day of the bioreactor run. Sheff-Pulse CD v1 also had a positive effect on cellular metabolism where it can be observed that lactate and ammonia levels were kept to low levels throughout the run (Figure 8). Most importantly Sheff-Pulse CD v1 had a strong effect on IgG titer. The levels of IgG increased by >120% by day 14 when compared to the other conditions (Figure 9). Due to the poor performance of the competitor control this Fed-batch work is being repeated in an effort to optimize the competitor product for this clone.

These products are prototypes and further work is required before they will be launched. Kerry expects to launch these products by Q4 2013.





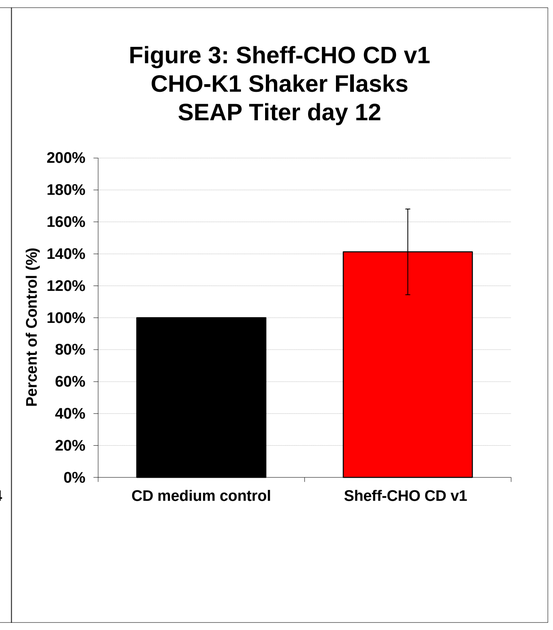
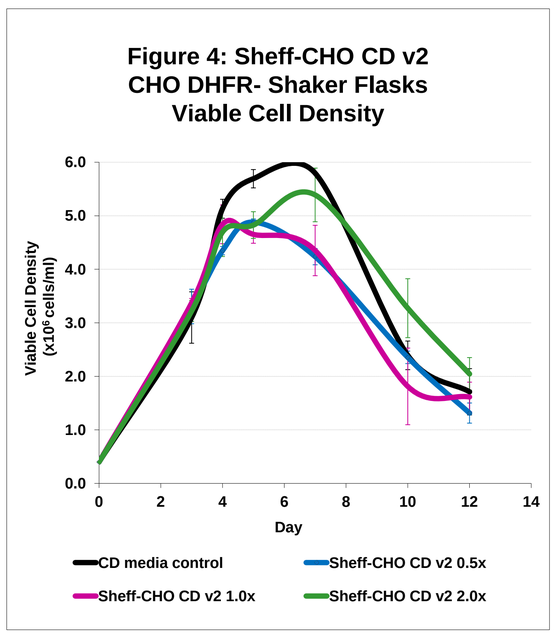
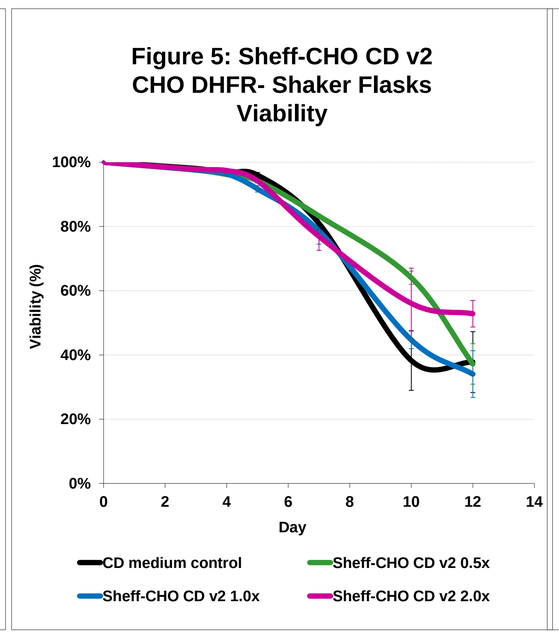


Figure 1, 2 and 3: *The impact of the Sheff-CHO CD on CHO-K1 was assessed in a commercially-available chemically defined CHO cell medium. The effects of Sheff-CHO CD v1 on viable cell density, culture viability and protein titer was assessed to determine overall culture health and productivity. Sheff-CHO CD v1 supplementation in basal media demonstrated a 40% improvement of product yield in CHO-K1 when compared to the basal media control.*





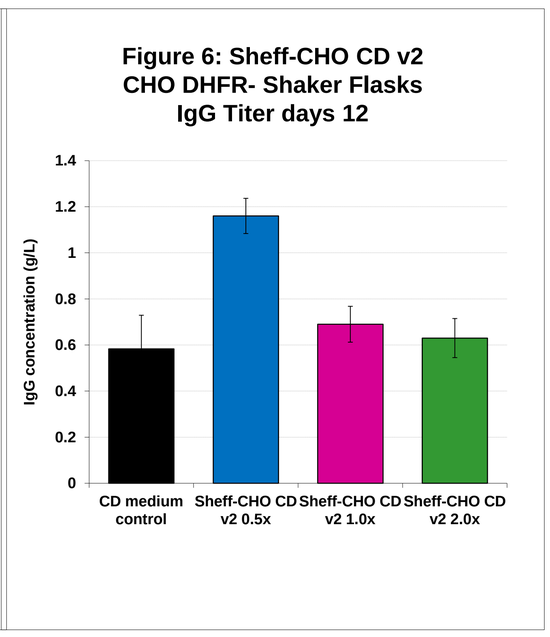
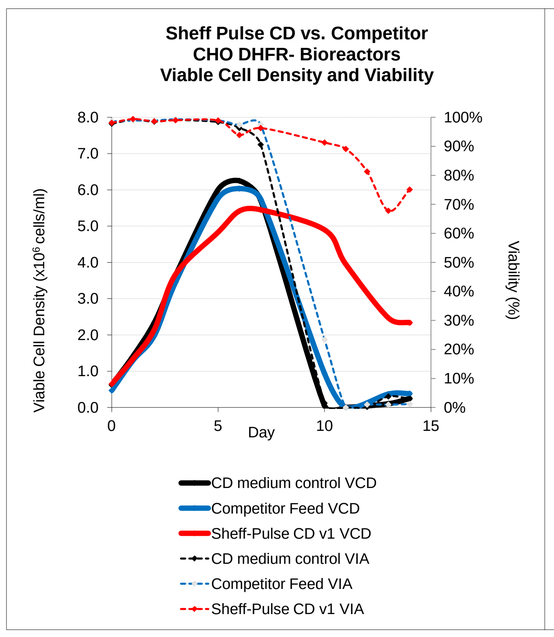
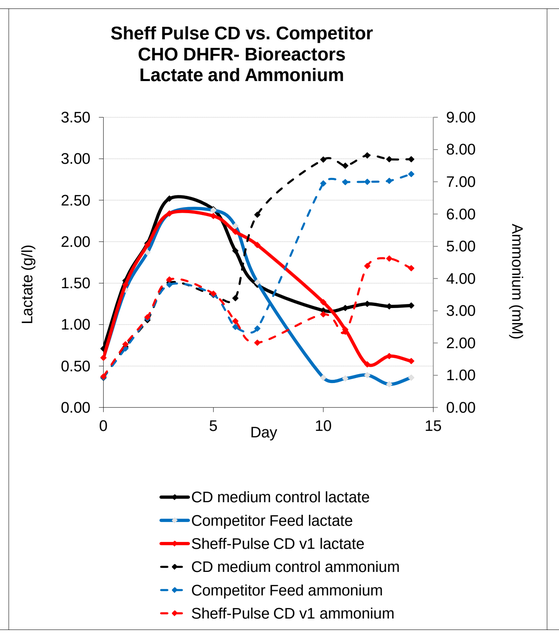


Figure 4, 5, and 6: *The impact of the Sheff-CHO CD v2 on CHO-DHFR-was assessed in a commercially-available chemically defined CHO cell medium. The effects of Sheff-CHO CD v2 on viable cell density, culture viability and protein titer was assessed to determine overall culture health and productivity. Interestingly Sheff-CHO CD v2 improved cell viability when utilized at higher dosages of 1.0 and 2.0x. However, supplementation of Sheff-CHO CD v2 at the lower dosage of 0.5x in CD basal media demonstrated a ~100% improvement in antibody product yield in CHO-DHFR- when compared to the CD basal media control.*





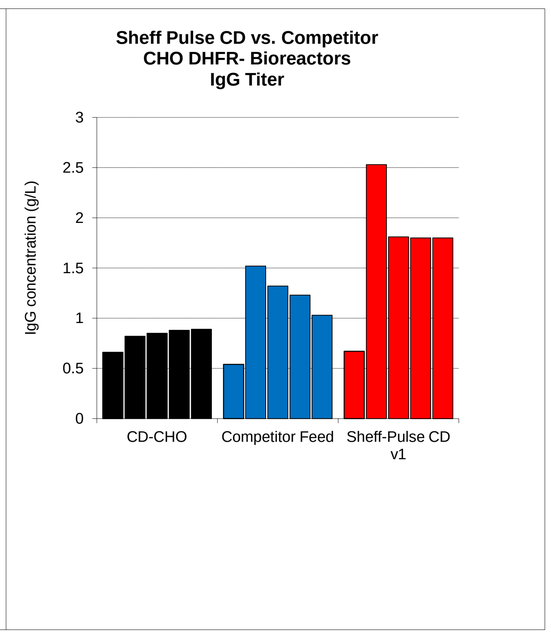


Figure 7, 8 and 9: *The growth and productivity improvements observed when supplementing with the Sheff-Pulse CD v1 feed system in a shake flask model were reproduced at the 1L bioreactor scale. Sheff-Pulse CD was fed on days 3, 5, 7, 9 and 12. Competitor supplement was fed according to manufacturer's recommendations. The Sheff-Pulse CD feed supplement was capable of extending the CHO DG44 growth curve beyond that of the commercially available, CHO specific CD medium control. On day 14, the Sheff-Pulse CD supplemented reactor maintained the highest cell density. Lactate and ammonium levels were much lower when the Sheff-Pulse CD was utilized than compared to the media and competitor control. IgG titer levels were significantly higher in the Sheff-Pulse supplemented reactor throughout the experiment, leading to almost double the final IgG concentration by day 14, when compared to CD media control.*