Improved Manufacturability of Fed-Batch Systems Employing Highly Concentrated Feeds

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ABSTRACT

Nutritionally complex feed supplements, particularly those that can satisfy metabolic demands of high-performing CHO cell culture, are essential for the successful optimization of present-day fed batch processes. An ideal nutrient component solution should be designed to facilitate manufacturability at the clinical and commercial production scale with regards to constraints on product dilution, fluid transfer and handling, and bioreactor working volume. All these criteria can be addressed through the utilization of a highly concentrated feed medium. To achieve this, traditional approaches for process development permit the introduction of substantial pH adjustments and multiple component subgroups. However, these practices can present additional complications. This study outlines our efforts towards the design of single-part highly concentrated feed supplements that do not require pH adjustment. These complete and balanced dry format feed media prototypes can be reconstituted rapidly in water and have achieved stable concentrations of 150-200g/L at neutral pH. Experiments were conducted confirming the feeds at 1X concentration delivered equivalent productivity of a monoclonal antibody in comparison with first generation commercial feed supplements, and when increased to 2-3X performance was improved by 20-100%. Glycosylation analysis on harvest samples containing the monoclonal antibody product showed that the predominant species was still G0F and that the major glycoforms were not changed as a result of feeding cultures with the new more concentrated prototypes. Additional case studies on multiple cell lines and processes will also be examined.

INTRODUCTION

Single-part dry format feed media for CHO bioprocesses that can be simply hydrated in reduced liquid volumes without the need for pH adjustment were developed through the use of novel and proprietary technology that allows for delivery of higher concentrations of difficult to solubilize components. This approach addresses several challenges associated with large scale fed-batch manufacturing, such as bioreactor working volume restrictions, safety concerns with large volumes of high pH solutions, lengthy fluid transfer times, and limited storage space. It also diminishes additional complications that can accompany pH-adjusted solutions, such as time consuming and complicated preparation, additional pH control management, supply chain pressures from use of short shelf life materials, and the inability to combine feed solutions without component precipitation. These materials can achieve stable concentrations of 150-200g/L at neutral pH after simple reconstitution in water resulting in solutions without excess osmolality associated with pH adjustment (Table 1).

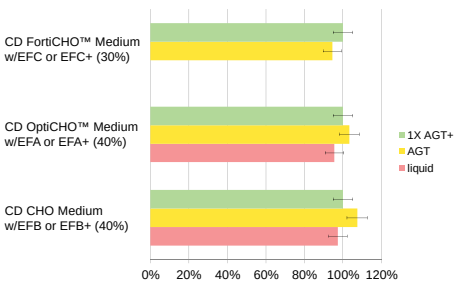
Table 1. Comparison against first generation feed media

		Level	g/L	Mix time (min)	pH	Osmo (mOsm/kg)
CHO CD EfficientFeed™ A AGT™ nutrient supplement	EFA	1X	65.1	65-80	7.1*	500
EfficientFeed™ A Plus AGT™ nutrient supplement	EFA+	1X	66.4	10	6.8	476
		3X	199.2	70	6.6	1567
CHO CD EfficientFeed™ B AGT™ nutrient supplement	EFB	1X	54.15	65-80	7.2*	400
EfficientFeed™ B Plus AGT™ nutrient supplement	EFB+	1X	52.7	20	7.0	361
		3X	158.1	45	6.8	1152
CD EfficientFeed™ C AGT™ nutrient supplement	EFC	1X	79.6	>65	7.0*	835
EfficientFeed™ C Plus AGT™ nutrient supplement	EFC+	1X	81.2	40	6.9	645
		2X	162.4	55	6.7	1365

* Indicates pH adjustment required

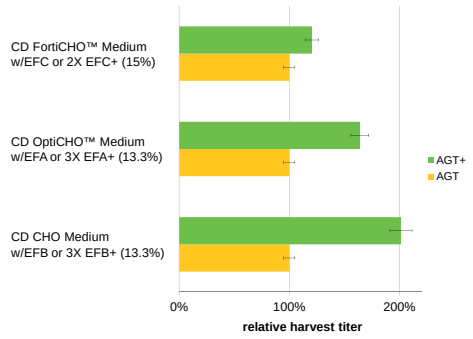
Six months of stability at 2-8°C in the dark has been established through analytical testing (UPLC) for both dry and liquid formats of each feed medium. In addition the liquid formats have been shown to be stable for one month at room temperature when protected from light. Development studies were conducted establishing comparability to first generation feed media at equivalent concentration (Figure 1) and superiority of highly concentrated options (Figure 2).

Figure 1. New feeds perform comparably to first gen feeds



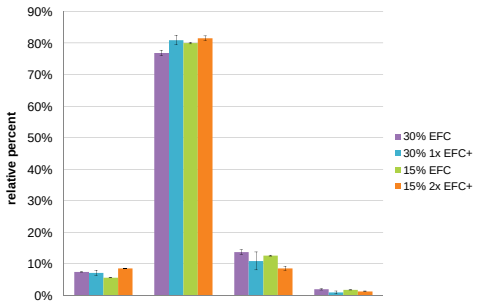
CHO DG44 cells expressing IgG were maintained in media containing 4mM L-glutamine. CD FortiCHO™ Medium was fed on days 5, 7, and 9 with 10% of either EFC or 1X EFC+, CD OptiCHO™ Medium was fed on days 5, 7, 9, and 11 with 10% of either EFA liquid, EFA AGT™ format, or 1X EFA+ AGT™ format, and CD CHO Medium was fed on days 5, 7, 9, and 11 with 10% of either EFB liquid, EFB AGT™ format, or 1X EFB+ AGT™ format. Cell culture performance is normalized to new feed results.

Figure 2. Highly concentrated feeds outperform first gen feeds



CHO DG44 cells expressing IgG were maintained in media containing 4mM L-glutamine. CD FortiCHO™ Medium was either fed with EFC at 10% on day 5 and 5% on day 7 or with 2X EFC+ at 5% on days 5, 7, and 9. CD OptiCHO™ Medium was either fed with EFA at 10% on day 5 and 3.3% on day 7 or with 3X EFA+ at 3.3% on days 5, 7, 9, and 11. CD CHO Medium was either fed with EFB at 10% on day 5 and 3.3% on day 7 or with 3X EFB+ at 3.3% on days 5, 7, 9, and 11. Cell culture performance data are normalized to original feed results. An example of glycan results for CD FortiCHO™ Medium from the previous work is shown in Figure 3.

Figure 3. Glycoforms comparable for different feeds



After the completion of development additional case studies were conducted to confirm these results in several cell lines.

MATERIALS AND METHODS

Case Study 1 (CS1): Two CHO-S clones expressing different antibodies were maintained in CD FortiCHO™ Medium and then inoculated into shake flasks. Cell cultures were fed on days 3, 7, and 10 with EFC or 1X EFC+ for a total of 45% of starting volume or with 2X EFC+ for a total of 22.5% of starting volume.

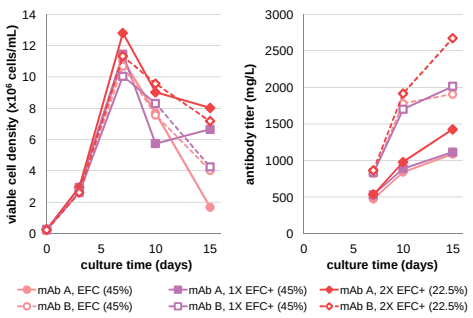
Case Study 2 (CS2): A recombinant CHO cell line was cultured in ambr15™ microbioreactors (TAP Biosystems) containing CD CHO Medium. Multiple conditions were fed on days 4, 6, 8, and 11 with either 12% EFA, 12% EFB, 6% EFA & 6% EFB, 12% 1X EFB+, 6% 1X EFB+, 6% 2X EFB+, or 6% 1X EFA+ & 6%1X EFB+.

Case Study 3 (CS3): CHO DG44 cells expressing IgG were maintained in CD OptiCHO™ Medium containing 6mM L-glutamine and then sequentially adapted into multiple test media containing 6mM L-glutamine. Vendor Media 1A and 2 did not respond well to sequential adaptation so instead they were directly adapted. Cell cultures were maintained in all media for 5 passages and were then inoculated into 55mL volumes in shake flasks containing 4mM L-glutamine. CD OptiCHO™ Medium was fed with 3X EFA+, CD CHO Medium was fed with 3X EFB+, and CD FortiCHO™ Medium was fed with 2X EFC+. Each condition was fed daily from day 4 to day 11 for 15% total. Vendor media cultures were fed with recommended solutions according to recommended protocols: Vendor 1 medium A was fed daily for 12 days with a pH neutral feed and a high-pH feed at a 10:1 ratio; Vendor 2 medium was fed on days 3, 5, 7, and 9 with a pH neutral feed and a high-pH cysteine/tyrosine feed (as recommended) at a 20:1 ratio; Vendor 3 medium was fed on days 1, 3, and 5 with a single pH neutral feed; Vendor 4 medium was fed on days 1 and 3 with a single pH neutral feed. Cultures were discarded after dropping below 50% viability.

Case Study 4 (CS4): A CHO cell line was cultured in 5L bioreactors containing CD CHO Medium for 18 days with a temperature shift, following a historical process that has been run multiple times (n=12). Feeds were delivered in three equal amounts on days 4, 6, and 8 and glucose was maintained above 2g/L. In the historical process, feeds comprise a combination of EFB, EFC, and FunctionMAX™ TiterEnhancer (FMTE) for a total of 33.3% of bioreactor working volume. The original EfficientFeed™ products were replaced with equimolar amounts of 3X EFB+ and 2X EFC+ and FMTE additions were maintained, allowing total additions to be reduced to 20% of bioreactor working volume. Process data were compared against historical results and product quality data from Protein A purified samples were compared against a fully purified reference control standard.

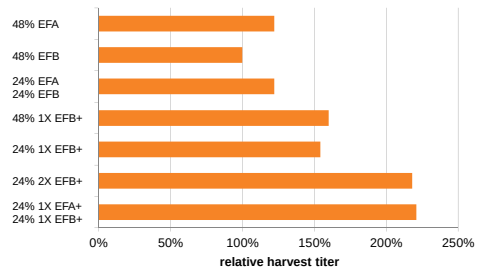
RESULTS

Figure 4 a & b. 30-40% improved productivity with highly concentrated feed (CS1)



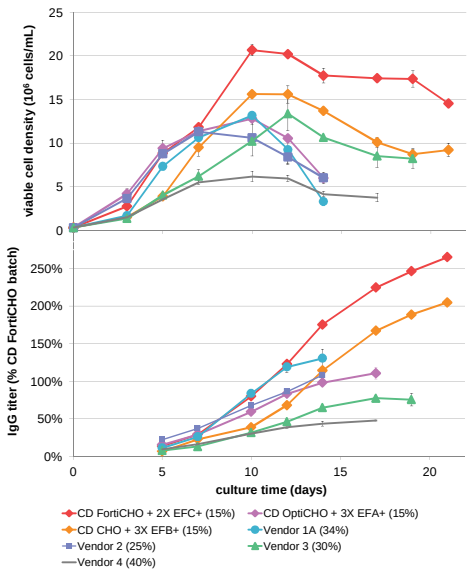
Peak cell densities (above) and viability profiles (not shown) were similar when comparing the EFC and EFC+ conditions. Final osmolality was comparable between EFC and 2X EFC+ (~420 mOsm/kg on average) and was lower for the 1X EFC+ conditions (~330 mOsm/kg on average). There was no discernible difference in titers between the EFC and 1X EFC+ conditions, and the half-volume feed of 2X EFC+ demonstrated a 30% titer improvement for antibody A and 40% titer improvement for antibody B when compared with EFC. The 2X EFC+ process also allows for unrestricted scale-up to manufacturing as the associated production bioreactors do not permit feeding in excess of 30% (23% working volume). In addition, the preparation of EFC+ liquid from dry format was observed to be significantly easier and faster, 45 minutes as opposed to 3 hours for the original feed.

Figure 5. 120% improved productivity with highly concentrated feeds (CS2)



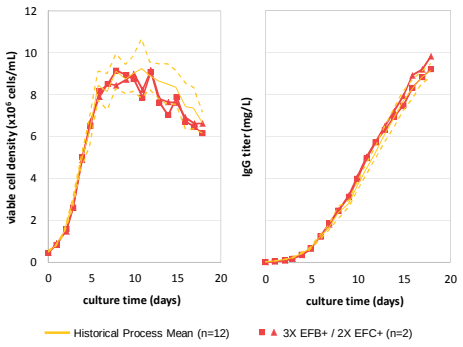
Use of 1X EFB+ and 2X EFB+ both substantially improved product titer over EFB (+60% and +120%). EFB+ was also effective in increasing peak cell density (not shown); 24% 1X EFB+ improved peak cell density by 65% over 24% EFB alone, and 24% 2X EFB+ improved it by 90%. Viability at harvest was also quite high for the conditions with EFB+ (>92%).

Figure 6 a & b. Comparison against alternative fed-batch strategies (CS3)



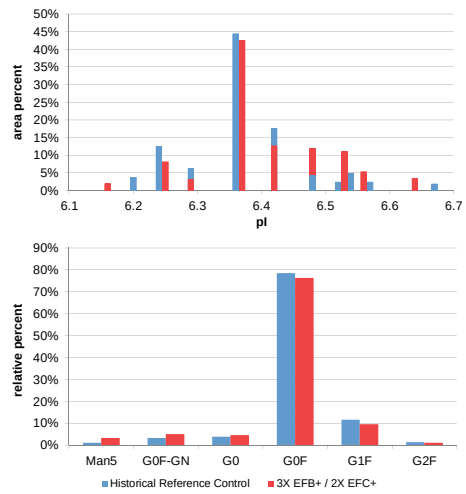
Results from Vendor 1 medium B and Vendor 5 medium are not shown as they did not achieve peak cell densities higher than $5x10^6$ viable cells per mL. After feeding was complete, osmolality data were compared on Day 14 and mostly they fell in the range of 320-410 mOsm/kg. Conditions outside the range include Vendor 1A (586 mOsm/kg), Vendor 2 (511 mOsm/kg) and Vendor 3 (274 mOsm/kg). Higher osmo values are likely the result of high-pH feed solutions.

Figure 7 a & b. Comparable growth and productivity in 5L bioreactor with less volume of highly concentrated feeds (CS4)



Both processes have consistent specific productivity and achieve >3g/L at harvest. Inclusion of FMTE may have already optimized productivity such that EFB+/EFC+ did not deliver an anticipated titer boost. The EFB+/EFC+ conditions had somewhat higher glucose levels and more lactate accumulation at the end of the run compared to historical control data (not shown). Osmolality was slightly higher after completion of feeding (25-50 mOsm/kg) in conditions with EFB+/EFC+, which was likely the result of less culture dilution but it remained under 400 mOsm/kg. Overall the results were determined by the evaluator to represent equivalent cell culture performance based on the historical control mean (solid yellow) and standard deviation (dashed yellow).

Figure 8 a & b. Comparable cIEF and glycoforms in 5L bioreactor with reduced feed volume (CS4)



Glycan results represent an average of two samples. No appreciable difference was observed versus reference control standard. Minor variation could be attributed to the samples not being fully purified and that the reference control standard was produced in a different process with low volumetric productivity. The evaluator did not consider differences to be significant.

CONCLUSIONS

- Lower volume addition of highly concentrated feeds provided up to 120% productivity increase
- Substantially easier and faster concentrated feed preparation
- Circumvented constraints to bioreactor working volume
- Dry and liquid formats stable for 6 months stored at refrigerated temperatures and when protected from light, 1 month at room temperature
- Comparable product quality to first generation feeds

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TRADEMARKS/LICENSING