

# Simplification of Fed-Batch Process for PER.C6® Cells in Bioreactors for Enhanced Monoclonal Antibody Production

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## Abstract

To attain the quantity of product required to meet the needs of their customers, manufacturers of monoclonal antibodies often use a fed batch bioreactor system for production. Each additional feed requirement increases the risk of contamination and makes the manufacturing process more complicated for those in charge of bioreactor operation. Contamination and manufacturing issues cost the company in time and money and can result in customer dissatisfaction. This project focused on minimizing the number of feeds added to the bioreactor while maintaining the benefit of the increased productivity. This reduces the risk of contamination and improves the ease of manufacturing.

The results were obtained using the PER.C6® cell line, which was derived from a human embryonic retinoblast cell and developed and licensed by Crucell™. It is used as an expression platform for the development and manufacture of therapeutic monoclonal antibodies (mAb). Utilizing optimized growth and production media for the PER.C6® cell line (EX-CELL™ 6GRO and EX-CELL™ 6PRO, respectively), and the newly formulated concentrated feed, we have developed a streamlined feed strategy for the bioreactor systems. The initial feed process for this cell line required 21 components to be combined at the time of feeding into two different formulations depending on which day they were fed. This feed process was significantly improved by developing a manufacturing method to produce a stable single concentrated feed from these components which could be manufactured in advance. Both feed methods yielded a significant increase in growth and productivity over non-fed cultures (> 45%), but the concentrated feed yielded an even greater increase in productivity over the non-fed cultures (63%). In utilizing the single-feed concentrate for the fed-batch process, the final result is not only a simplified fed batch strategy suitable for recombinant protein manufacturing, but also a further increase in productivity.

## Introduction

Industry continually pushes the cell lines that they use for manufacturing to further productivity limits. Every aspect of the process is scrutinized and optimized from the development of the cell line, the medium, the process used for production and the downstream processing. Every one of these processes is important and lends itself to improvement and increased productivity. Using a fed-batch process has historically shown to have a very significant effect on productivity and longevity of the culture. The feed that is supplied, the timing of the feed and the process controls to support the feed all determine if the fed-batch process is a success. Once the process is established, simplifying the process to make it manufacturing-friendly and robust is the next step.

The PER.C6® cell line was developed to be a very high producing cell line for monoclonal antibodies. Media have been optimized for growth and productivity, but a fed-batch process has remained complex. Our focus, in collaboration with Crucell™, was to simplify the fed-batch process by developing a manufacturing method for the complex feed and demonstrating performance in fed-batch bioreactor systems.

## Materials and Methods

### Cell Culture

A suspension PER.C6® IgG producing cell line, EPCAM IgG, subclone 088-009 (Crucell Holland B.V., The Netherlands), was used for the confirmation of the modified feed concentrate in the bioreactor systems. Stock cultures were maintained and expanded in EX-CELL™ 6GRO medium (Item No. 14641C) supplemented with 6 mM L-glutamine in Corning shaker flasks, 120 rpm, 37 °C, and 5% CO<sub>2</sub>. The seed train consisted of seeding cells at 2 × 10<sup>5</sup> and expanded every 2 – 3 days. The final cultures were pooled prior to inoculation into 3 L bioreactors with EX-CELL™ 6PRO (Item No. 14642C) medium for production.

### Bioreactor Set Up and Parameters

The bioreactors used were 3 L Biobundle™ systems (Applikon® Biotechnology, Schiedam, Holland). Cells were seeded at 7.5 × 10<sup>5</sup>/mL in 1.5 L working volumes using 350 mL of cells and 1150 mL fresh EX-CELL™ 6PRO medium with 6 mM L-glutamine and maintained for 13 days. The operating parameters were as follows: 50% DO; agitation at 100 rpm on day 0, 125 rpm on day 2, 150 rpm on day 6; 37 °C; pH was not controlled.

### Feeding Strategies

There were two feeding strategies employed for comparison purposes; control bioreactors were not fed. The cells were fed in bioreactors on days 6, 8 and 10 with the amount of the feed solution increased on days 8 and 10 based on the projected cell density. For the first feed strategy (referred to as the "Multi-part feed"), the feed consisted of 21 components that had to be combined just prior to addition to the reactors. The concentrations of two of the components in this feed strategy were different for the day 6 feed as compared to the day 8 and 10 feed. For the second feed strategy (referred to as the "Concentrated feed"), the feed was formulated as a one-part concentrate (imMEDIATE ADVANTAGE™ 65741), packaged in 350 mL BIOEAZE™ EVA bags (Item No. 522B), sterile connected to the reactor systems and used for the entire run. Both feeds contained glucose and L-glutamine; additional amounts were not required.

### Assessments and Analytical Methods

The cultures were sampled daily with by removing 10 mL from the bioreactors. The samples from days 8 and 13 were also used to analyze the cultures for productivity (day 8 data not shown). The cells were counted daily between days 0 – 13, with the exception of day 4, using the Cedex Automated Cell Analyzer (Innovatis AG, Bielefeld, Germany). Metabolic consumption of L-glutamine and glucose and production of ammonia and lactate were measured offline with the BioProfile® 400 (Nova Biomedical Corp., Waltham, MA). The IgG was measured by HPLC using a Zorbax 450 Column (Agilent Technologies, Santa Clara, CA), a Waters Alliance 2695 Quaternary Separation Module and a 996 PDA Detector (Waters Corporation, Milford, MA).

## Results and Discussion

The original fed batch strategy that was developed for maximizing IgG productivity in PER.C6® cell lines was very complicated but yielded a >45% increase in productivity. However, the complexity of formulating the feeds for this process precluded this process from manufacturability. Therefore, our goal was to enable the feed strategy to better align with a manufacturing process while also maintaining the high productivity conferred by the feed.

The first step was to determine how concentrated each of the 21 feed components could be made and remain soluble in a given pH range. This solubility information yielded a starting point of 25×. Feasibility of a concentrate manufactured in this way was performed, however there remained solubility issues.

Given these issues, the salts were either removed or reduced from the concentrated feed. It had been determined that, for performance reasons, there are components in this group which need to be maintained in the feed. Therefore, these were reduced to 1× concentrations so that they were present for the cells to utilize during their extended growth period, and the feed was able to be manufactured. This formulation modification yielded a concentrated feed (imMEDIATE ADVANTAGE™ 65741) that would remain soluble and stable for greater than 30 days at a pH of 7.2 at 2 to 4 °C, ambient temperature, and 37 °C.

The feeds were then testing in bioreactors to determine if the concentrated feed would perform comparably to the original multi-part feed. The bioreactors were inoculated from the same pool of cells to assure that the only variable was the feeding strategy. In order to maintain the DO at 50%, it was necessary to increase the agitation rate on day 2 to 125 rpm and again on day 6 to 150 rpm due to the high oxygen demand of the cells. Aliquots from the daily samples were retained and analyzed for productivity on days 8 and 13 in order to compare productivity titers using the two feeding strategies. Figures 1 and 2 show the growth, viability and productivity from the reactors. The graphs indicate that the concentrated feed not only yields increased productivity but also supports higher cell densities with less variability than the multi-part feed. The consumption of glucose and L-glutamine and production of lactate and ammonia were measured using the NOVA BioProfile. This data is shown in Figures 3 through 6. These consumption and production rates trend very similarly using the two feeding strategies suggesting that there are no significant metabolic differences between using the two strategies.

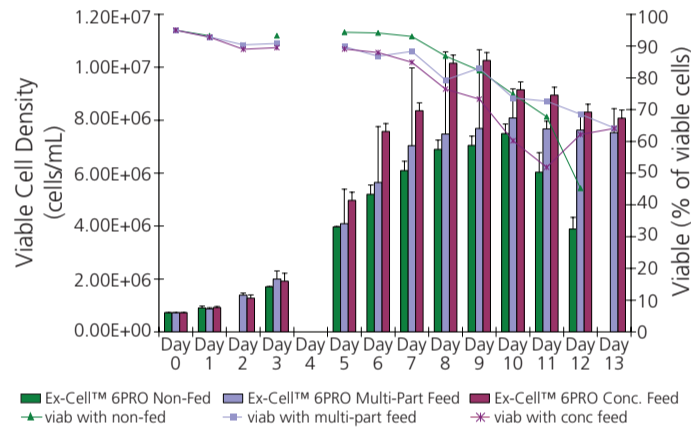


Figure 1: Average growth and viability using EX-CELL™ 6PRO Medium in bioreactors comparing non-fed cultures with those using either the multi-part feed or the concentrated feed. (imMEDIATE ADVANTAGE™ 65741)

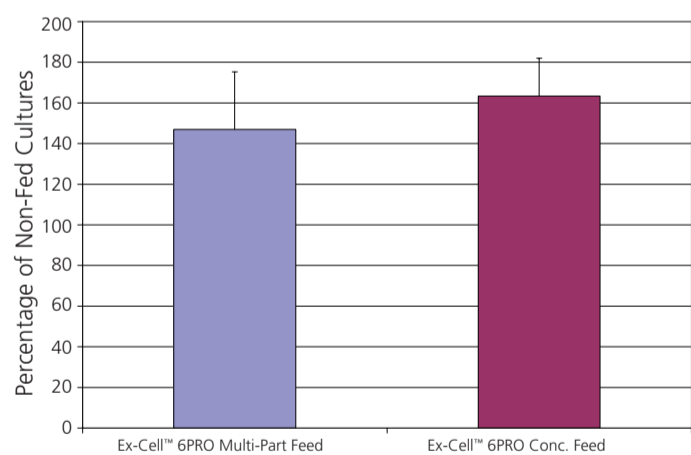


Figure 2: Average increase in productivity using EX-CELL™ 6PRO Medium and either the multi-part feed or the concentrated feed; expressed relative to non-fed bioreactor cultures

### Average L-glutamine Consumption

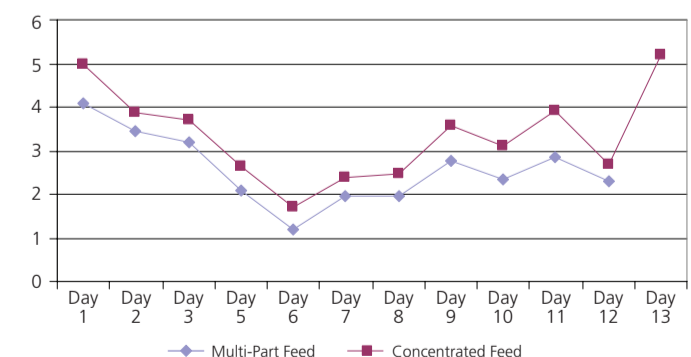


Figure 3: Average L-glutamine consumption in bioreactor cultures using either the multi-part feed or the concentrated feed (imMEDIATE ADVANTAGE™ 65741) in conjunction with EX-CELL™ 6PRO Medium

### Average Ammonia Production

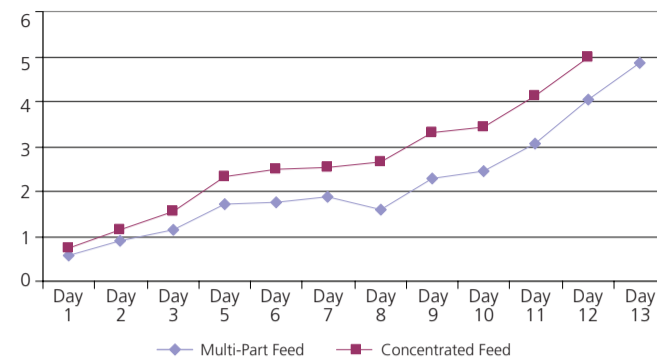


Figure 4: Average ammonia production in bioreactor cultures using either the multi-part feed or the concentrated feed (imMEDIATE ADVANTAGE™ 65741) in conjunction with EX-CELL™ 6PRO Medium

### Average Glucose Consumption

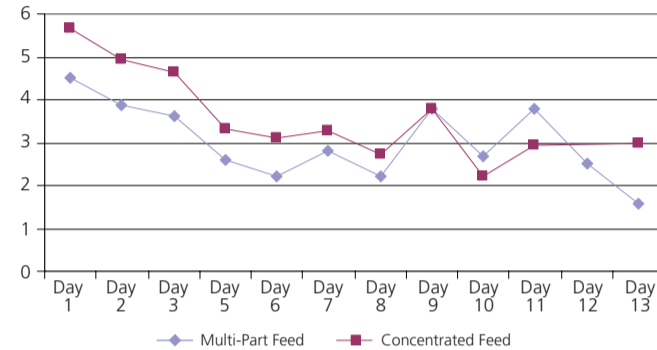


Figure 5: Average glucose consumption in bioreactor cultures using either the multi-part feed or the concentrated feed (imMEDIATE ADVANTAGE™ 65741) in conjunction with EX-CELL™ 6PRO Medium

### Average Lactate Production

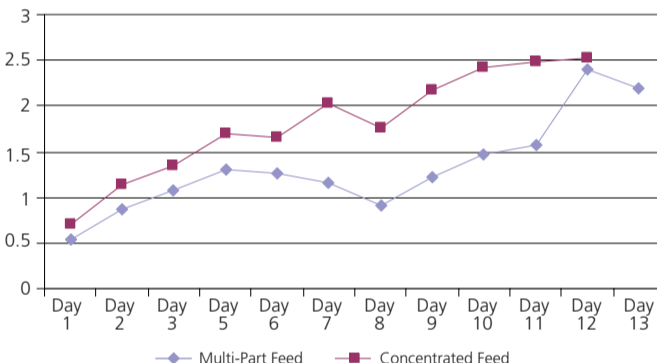


Figure 6: Average lactate production in bioreactor cultures using with the multi-part feed or the concentrated feed (imMEDIATE ADVANTAGE™ 65741) in conjunction with EX-CELL™ 6PRO Medium

## Conclusions

Both the multi-part feed as well as the concentrated feed yielded an increase in productivity over the non-fed conditions when cultures were maintained in bench scale bioreactors. However, the concentrated feed yielded higher productivity than the multi-part feed (63% and 45% respectively), as well as reduced variability in growth. The development of a manufacturing method for the complex feed resulted in a simplification of the feed strategy. This, and the fact that the feed has demonstrated shelf life stability, enables the concentrated feed (imMEDIATE ADVANTAGE™ 65741) to be used in a recombinant protein manufacturing process.

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