Optimization and Evaluation of Perfusion Medium in High Cell Density Mammalian Cell Culture Systems.

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ABSTRACT

Continuous manufacturing has been investigated in both academia and industry as an alternative to batch manufacturing and has been increasingly accepted in the past decade. As a well-recognized method for continuous production of biomolecules expressed in CHO cell culture, perfusion processes are known to have certain advantages over batch and fed-batch processes. The major advantage of perfusion processes is that a favorable environment is provided to cells through the continuous removal of waste by-products and the constant replenishment with fresh media. This environment enables high viable cell densities over long periods of time, resulting in high total productivity. The top candidate (PM8) of a CHO media panel consisting of ten candidates was selected by using several established high-throughput small-scale models and bench-scale bioreactors with the Mini-BioSep™ acoustic perfusion system, and optimized focusing on factors such as amino acids, vitamins, trace metal elements, and other components through Design of Experiments (DoE) methodology. The perfusion media were further optimized through a multi-step DoE study using a refined high-throughput small-scale model in ambr®15 micro-bioreactors. In the optimized conditions, the viable cell density peaked at 55 million viable cells per milliliter (vc/mL) and the cell viability was maintained above 90% throughout the duration of the run with a medium exchange strategy of 1 reactor volume per day (RVD) in an 11 day cell culture assay. The lead prototype perfusion medium resulted in daily IgG titer that represented an increase of 200% over the titer achieved with a commercially available medium designed for fed-batch culture. The CHO media panel was also evaluated by alpha testers indicating the same top candidate of the CHO media panel, PM8 in terms of daily peak titers. The viable cell density was achieved over 100 million vc/mL with a cell specific perfusion rate (CSPR) of 22pL/cell/day in a mini-bioreactor with a tangential flow filtration (TFF) system. The volumetric productivity of 3 g/L/day was reached. These media, which have been specifically developed and optimized for perfusion applications conducted at a low medium exchange rate, will enable perfusion processes to be more efficient and reduce the media cost per gram of antibody produced.

INTRODUCTION

A panel of diverse perfusion media was specifically designed to support high density continuous recombinant CHO cell culture with high yield. The media are chemically-defined, protein-free, animal origin-free and growth factor-free. Cells were adapted to the perfusion media panel for at least 5 passages. A refined high-throughput small-scale model in ambr 15 microbioreactors was applied to further optimize the top candidate formulation. During the experiments, samples were collected daily. Cell density and viability were measured by Vi-Cell™ XR Cell Counter (Beckman Coulter). Metabolites and product titers were analyzed by Cedex Bio HT Analyzer (Roche) and High Performance Liquid Chromatography (HPLC), respectively. Verification studies to confirm the optimized prototype medium in small-scale perfusion bioreactors are underway and the best candidate will be scaled up to HyPerforma™ Single-Use Bioreactors (SUBs).

RESULTS

Bench-scale Bioreactors with Mini-BioSep Acoustic Perfusion System

Figure 1. Bioreactor Runs: Cell Growth, Viability, and Titer

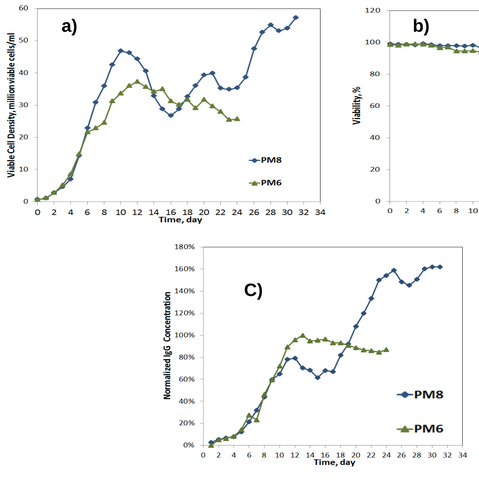


Figure 1. Cells were inoculated at 0.3 million vc/mL in bench-scale bioreactors using the Mini-BioSep acoustic perfusion system at 37°C, 40% dissolved oxygen (DO), and 150 RPM with a medium exchange rate of 1 RVD starting at D3. a) Cell growth, b) Viability, c) Titer. PM8 was found to be the best candidate based on the peak titers.

Figure 2. Pathway Analysis

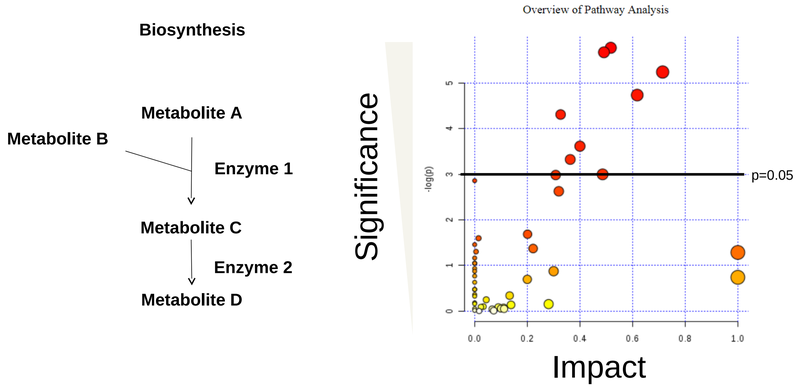


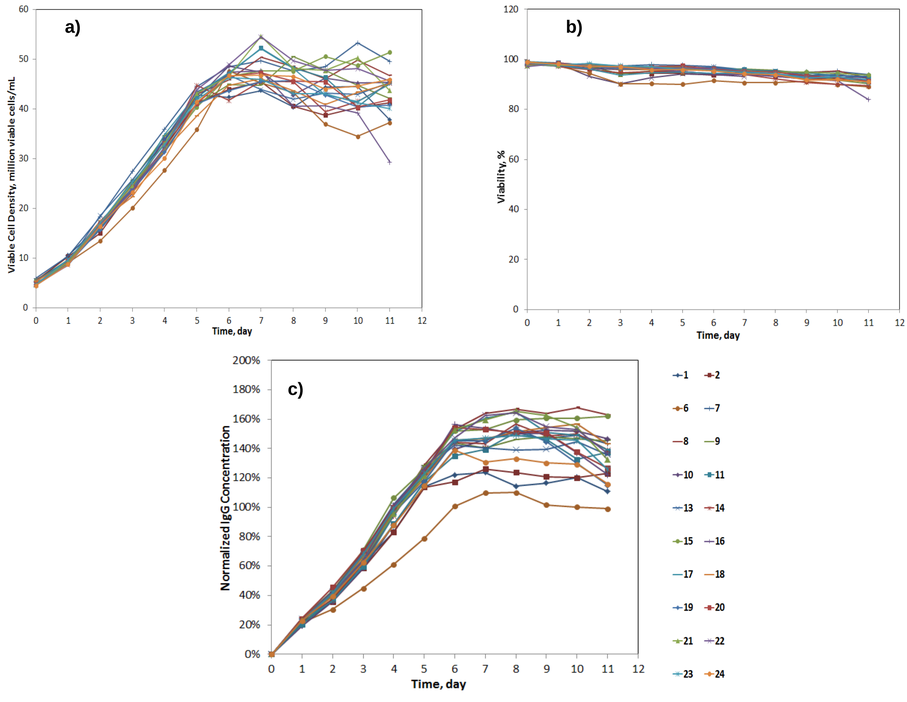
Figure 2: Advanced Cell Signature Analysis (ACSA) identifies critical pathways through proteomic and metabolomic approaches. Evaluation of PM8 versus Dynamis™ in a small – scale perfusion model in the ambr 15 identified metabolites which changed in relative abundance between the two formulations. Those components were then rated based on significance and impact in order to identify possible targets in a rational medium design.

High-throughput Small-scale Model in ambr 15

Figure 3. First DoE experiment: Cell Growth, Viability, and Titer

Figure 3. A two-level partial fractional factorial design (2^{5-1}) with four center points was applied for the first DoE experiment. Based on the statistical analysis, the conditions that showed positive effects on titer were selected (indicated by condition number). Cells were inoculated at 5 million vc/mL in ambr 15 microbioreactors at 37°C, 40% DO, and 1000 rpm with a medium exchange rate of 1 RVD starting at D3. a) Cell growth, b) Viability, c) Titer are shown. The viable cell densities reached up to 40 million vc/mL, and the highest IgG titer was 176% of the peak titer of Dynamis™ Medium.

Figure 4. Second DoE experiment: Cell Growth, Viability, and Titer



Figuer 4. A two-level partial fractional factorial design (2^{5-1}) with four center points was used for the second DoE experiments. Based on the statistical analysis, the condition that showed positive effects on titer was selected (indicated by condition numbers). Cells were inoculated at 5 million vc/mL in amb 15 microbioreactors at 37°C, 40% dissolved oxygen (DO), and 1000 rpm with a medium exchange rate of 1 RVD starting at D3. a) Cell growth, b) Viability, c) Titer. Viable cell densities of 55 million vc/mL were obtained an the peak IgG titer represented an increase of ~70% over the titer of best condition of the first DoE.

Figure 5. Overall improvement of Final Formulation over Dynamis

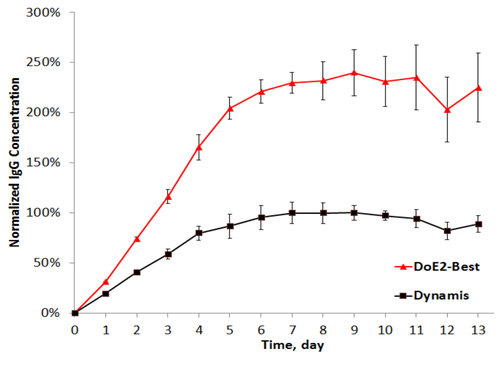


Figure 5. Verification of performance gains was conducted in tryplicate. Cells were inoculated at 5 million vc/mL in ambr 15 microbioreactors at 37°C, 40% dissolved oxygen (DO), and 1000 rpm with a medium exchange rate of 1 RVD starting at D3. Peak IgG titer represented an increase of 2.5x increase in titer achieved by Dynamis.

CONCLUSIONS

We tested several prototype media and used a rational DoE methodology to optimize a lead candidate based on specific design inputs to promote high density and high productivity perfusion culture. A small-scale model was established using the ambr 15 microbioreactor system with a 1 RVD medium exchange protocol. The peak IgG titer of the final optimized medium represented an increase of 240% above the peak titer achieved with the leading fed-batch medium, Dynamis. The results highlight that substantial gains in VCD and titer can be achieved when the cell culture medium is optimized specifically to provide the optimal nutritional balance necessary for a perfusion process.

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TRADEMARKS