Transcriptome analysis in high-producing CHO cell cultures: Strategies to design high-performing cell culture media

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Abstract

The present study investigates the beneficial effect of spiking HyClone™ ActiPro™ basal medium with HyClone Cell Boost™ 7a and Cell Boost 7b feed supplements on growth and productivity of a recombinant Chinese hamster ovary (CHO) cell line. To evaluate the impact of feed-spiking compared with cultivation in basal medium only, the cell line was grown in bioreactors under controlled conditions to determine cell-specific metabolic rates, nutrient consumption, and byproduct accumulation over the process time. Transcriptome analysis of the cultivated cells, using microarrays on four consecutive days to investigate differential gene expression, revealed the beneficial effect of feed-spiking compared with cells grown in basal medium.

Introduction

In lab-scale processes, when high amounts of product should be generated in a short period of time, simple equipment is often used in batch or fed-batch mode. Batch cultures are fairly simple and fast processes, but yield relatively low mAb titers. This finding is due to rather short cellular growth and production phases, accompanied by nutrient depletion and by-product accumulation. In fed-batch cultivation, concentrated medium is added to replenish nutrients. This increases cell concentrations, process duration, and ultimately recombinant product titers, but such processes are more complex as well as time and labor intensive. In search of a method that allows the generation of higher volumetric yields in a simple, non laborious batchoperated culture, we added concentrated Cell Boost 7a and 7b feed supplements only once to the ActiPro basal medium at the very beginning of a normal batch culture. The generated formulation was optimized to provide the maximum of nutrients to the cells before negatively affecting growth and productivity. These so-called feed-spiked batches exhibited almost two-fold higher cell-specific antibody production and boosted volumetric productivities approximately three-fold compared with the batch control cultivated in basal medium only. We applied microarrays to explore genes that could possibly be associated with the enhanced specific productivity.

Materials and methods

- Parallel batch cultivation of a CHO cell line in stirred-tank bioreactors (DASGIP™, CellFerm-Pro™, Eppendorf) at 37°C, pH 7.0, 30% DO.
- Model cell line: mAb-expressing CHO DG44 (licensed from Cellca GmbH)
- Cultivation media:
  - Cultures in basal medium: ActiPro (GE Healthcare) supplemented with glutamine
  - Cultures in feed-spiked medium: as cultures in basal medium, with additional supplementation with 7% Cell Boost 7a and 0.7% Cell Boost 7b (GE Healthcare)
- Analytics: cell concentration, viability, mAb concentration, selected metabolites, osmolality, amino acids
- Product quality: glycosylation, molecular size distribution, protein charge heterogeneity
- Transcriptome analysis by microarray technology: mRNA isolated daily was analyzed on a microarray platform, the 8 × 60 k design (Agilent) and statistically data analyzed (GeneSpring™). Gene ontology (GO) classification, pathway enrichment analyses (WebGestalt), and Gene set enrichment analysis (GSEA) were used to determine statistically significant differences between control and treatment condition in functional gene sets (C2) of the Broad Institute using the Molecular Signatures Database (MSigDB, now v. 5.1) (1, 2).

Results and discussion

Biologic experiments and impact on process performance

Both basal and feed-spiked processes lasted for seven days with viabilities above 95% until Day 6. On day seven, a sharp decline in viability indicated the end of the batch process (Fig 1A). In feed-spiked medium, cells initially grew slower but reached almost twice as high peak cell concentrations (17.6×10^6 c/mL) than in basal medium only (9.79×10^6 c/mL). Remarkably, the integral of the viable cell concentration over the total process time (viable cumulative cell days [VCCD]) was similar between both process strategies (Fig 1C). While mAb production plateaued after Day 4 in basal medium only (final titer 0.8 g/L), a continuous increase to three-fold higher final titers (2.4 g/L) was observed in feed-spiked medium (Fig 1B). The higher titers could be attributed to generally higher cell-specific productivities (qP), which remained rather constant (~ 70 pg/cell/day) in feed-spiked cultures. In basal medium, the qP continuously dropped by 20% (Day 0 to 3), 50% (Day 4), and > 90% (Day 5 to 7) from 70 to 10 pg/cell/day in basal medium cultures. In average, the qP was 70% higher in feed-spiked cultures (Fig 1D).

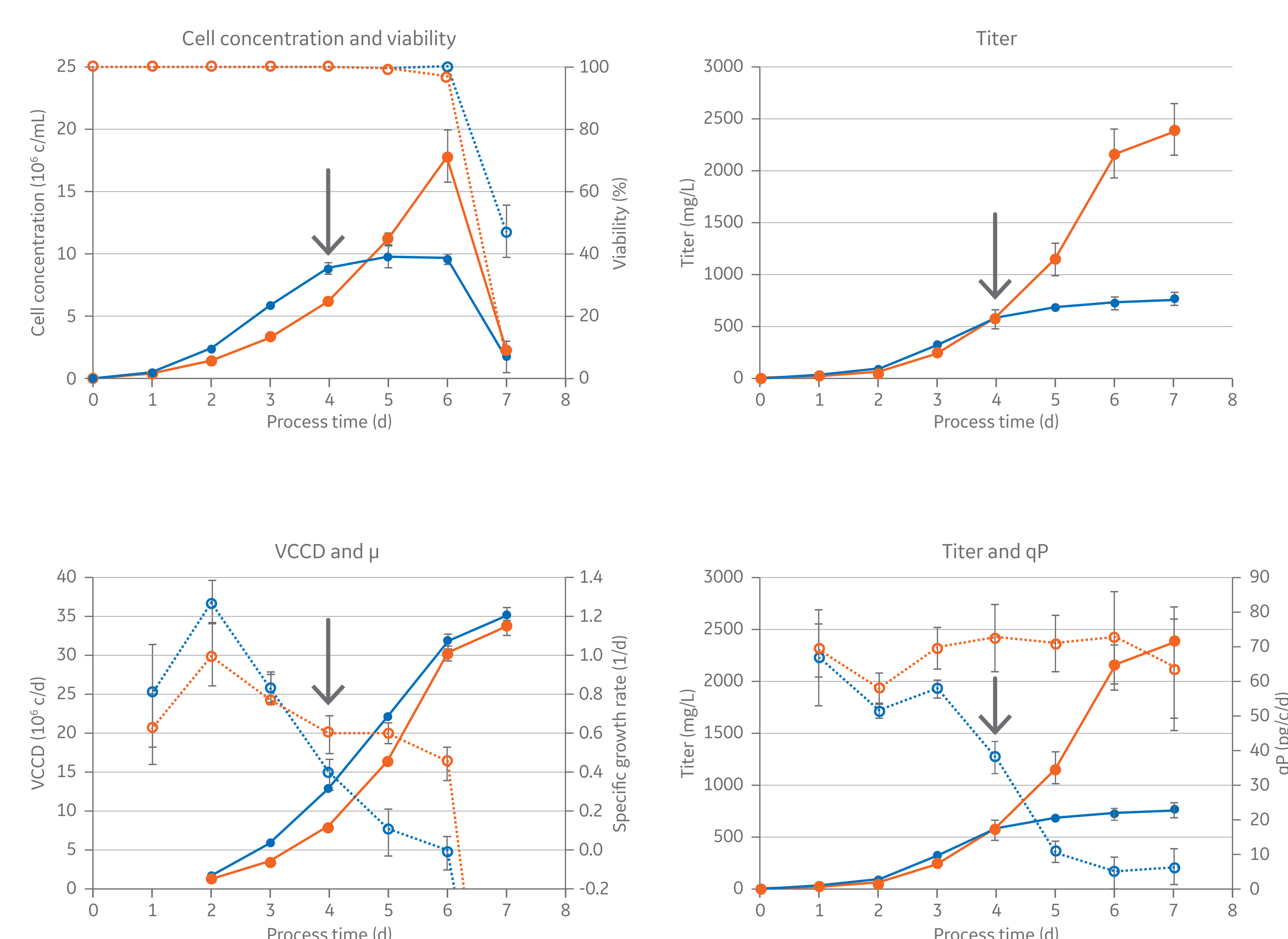

Fig 1. Process performance of basal medum (blue) and feed-spiked (red) bioreactor batch cultures: (A) cell concentrations and viability, (B) antibody concentrations, (C) viable cumulative cell days and specific growth rate, and (D) antibody concentrations and cell-specific productivity. Error bars indicate standard deviation from three independent experiments. The black arrows on Day 4 indicate the beginning of decreasing cell-specific productivities and lower cell-specific growth rates in basal medium cultures.

Concentrations and cell-specific rates of most of the analyzed key metabolites and amino acids were not conspicuous (data not shown). It appeared as if the applied nutrient cocktail of concentrated feed supplements enabled feed-spiked cultures to switch to a self-fueling energy metabolism (data not shown) and which allowed the cells to remain in a proliferative state in which mAb expression continued at constantly high rates.

Antibody product quality

Quality attributes of mAb produced by cells grown in basal or feed-spiked medium was similar (Fig 2). Therefore, feed-spiking boosted mAb yields by a factor of three with only minor impact on the quality of the expressed product.

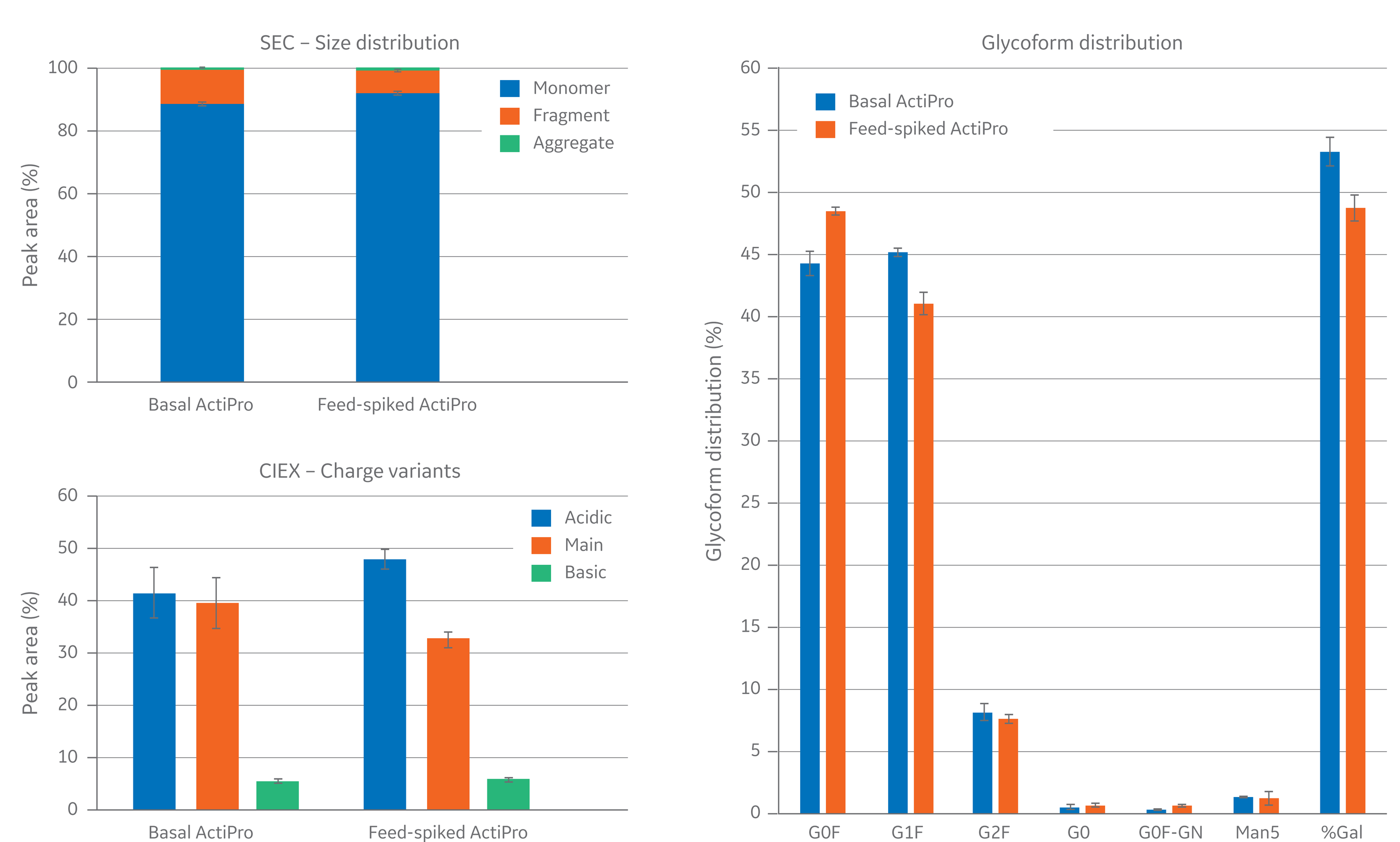

Fig 2. Key mAb product quality attributes: (A) molecular size distribution, (B) protein charge heterogeneity, and (C) glycoform distribution of harvested cell culture supernatant from the bioreactors. Error bars indicate standard deviation from three independent experiments.

Statistical analysis of differential gene expression

In total, 24 microarrays were processed from four different days (Day 3, 4, 5, 6), with biological triplicate for each sample (3 × basal; 3 × feed-spiked) and two technical replicates for each condition. Each microarray contained an average of 30 551 gene transcripts and variants. About ~ 60% (18 112) was used for statistical analysis.

Intersections of up- and down-regulated genes from Days 4 to 6 are visualized in Venn diagrams (Fig 3). In total, 332 individual genes were commonly up-regulated in feed-spiked medium from Day 4 until Day 6. During the same period the number of down-regulated genes was less than half (157 transcripts).

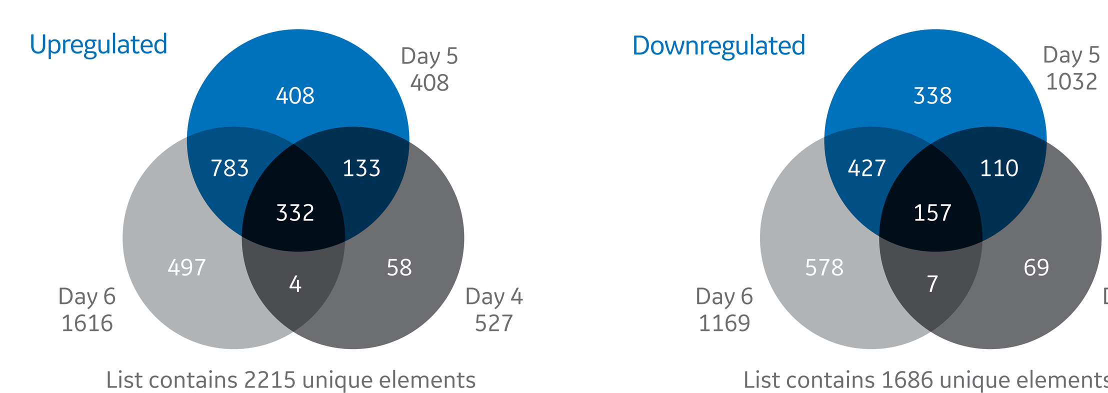

Fig 3. Venn diagrams of up- and down-regulated genes on Days 4, 5, and 6. Due to short gene lists, differentially expressed genes of Day 3 were not included.

The number of differentially expressed genes increased steadily when the qP dropped in basal medium cultures between Days 4 to 6. This finding suggests that the genes that accounted for the observed benefit of feed-spiked cultures were continuously differentially expressed throughout the cultivation and were considered relevant to explain higher cell-specific productivities in feed-spiked cultures.

The identified GO terms for the selected genes indicated a more active proliferative state for feed-spiked cultures (data not shown). The top GO terms significantly related to cell cycle and primary metabolism, cellular division, as well as nucleobase formation or regulation. Furthermore, GSEA revealed several significantly enriched set of genes related to gene transcription, DNA replication and repair, cell growth and proliferation, as well as inhibition of apoptosis in feed-spiked cultures. Thus, feed-spiking increased the proliferative activity of cultivated cells. Several of the identified genes appear as promising targets for cell line engineering, but have not yet been described in relation to high-producing recombinant cell lines and will need to be evaluated in future studies.

References

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Conclusion

Feed-spiking of basal medium is a convenient and easy way to considerably increase product concentrations in a simple batch culture. Differential gene expression revealed genes that appear important for high cell-specific production rates, and this knowledge can be leveraged into cell line engineering approaches or the design of high producing CHO cell media. In the latter case, a maximized supply of nutrients enabled a self-fueling energy metabolism (data not shown) and allowed mAb expression at constantly high rates. The described results were shown to be applicable to three additional recombinant mAb-expressing cell lines including CHO DG44, CHO-S and CHO-K1 (data not shown).

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