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The relevance of cell size in a CHO fed batch process: Metabolic and transcriptomic characterization

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THE RELEVANCE OF CELL SIZE IN A CHO FED BATCH PROCESS

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Background
The growth profile of a CHO cell fed-batch process can in general be divided into a growth phase followed by a stationary (non-growth) phase and a cell death phase. In a previous study an additional phase was observed between the cell proliferation and stationary phase during which cell numbers remained constant and cell size increased. The relevance of this cell size increase for the process performance as well as the cause for this shift in cell growth is, however, not clear.

Objective
Establish the relevance of the switch from cell proliferation to cell size increase for fed-batch processes, obtain more insight in the physiological changes that occur and understand the underlying mechanisms that cause these changes.

Methods
- CHO-K1 clone: BC-P producing an IgG 1
- TriPLICATE fed-batch cultures in 10 L bioreactors (Sartorius Stedim)
- DO: 50%, pH: 7.2±0.05, T: 37 °C, N: 200 RPM.
- ActiCHO-P + ActiCHO feed A&B (GE Healthcare)
- Daily bolus feeding: 4.5% feed A + 0.45% feed B, glucose top-up to 28 mM when lower than 18 mM.
- Metabolic flux modelling: Cobra toolbox for Matlab
- Transcriptomics: Affymetrix GeneChip™ CHO Gene 2.1 ST Arrays (Affymetrix, Santa Clara, USA)
- Cell cycle analysis: CycleTest™ Plus DNA Reagent Kit (BD Biosciences) & BD Accuri™ C6 Flow Cytometer (BD Biosciences).

Results

Figure 1. Total viable cell number and total viable cell volume in the bioreactor. Top photos: Cells stained with bodipy (lipids)
- Day 4 to 6 cell proliferation stops and cells start increasing in size.
- Cells are arrested in the G1 phase.
- Lipid accumulation in the G1 phase.

Figure 2. Cell cycle distribution from day 4, 5, 6 and 9.

Figure 3. Specific productivity in the NI and SI phase expressed per cell and per cell volume.

Figure 4. Cell specific productivity as a function of cell volume.

Figure 5. Metabolic flux distribution (mmol/109 cells/day) in the NI and SI phase. Green values indicate different fluxes whereas the red values indicate overlapping values based on flux variability analysis.

Figure 6. Transcriptional regulation of pathways influencing cell size. SI phase compared to NI phase.

Conclusions
- Cell size increase due to cell cycle arrest and continued growth.
  - Arrest mainly in the G1 phase
  - Down regulation cyclins and cyclin dependent kinases
  - Upregulation mTOR activity
- Ratio of product to cellular protein increases from 1.5 to 5%.
- Yield of product on oxygen increases with a factor 1.5.

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