TRANSCRIPTOMICS AND MODELLING TO UNDERSTAND THE BENEFITS OF LOW PERFUSION RATE

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The reduction of the medium renewal rate is very important for perfusion processes. However, the minimization of cell specific perfusion rate (CSPR) should not compromise the cell specific productivity of the antibody (mAb) or its quality. We investigated the effect of low CSPR on the cell phenotype and the mechanisms that allowed a very low CSPR of 10 pL/cell/day to retain the mAb productivity. For this, a perfusion culture of GS-CHO-K1 cells expressing Trastuzumab was performed in ActiPro medium with Cell Boost 7a/7b (Cytiva). Three consecutive steady-states at CSPRs 40, 20 and 10 pL/cell/day (CSPR40, CSPR 20, CSPR 10) were performed while the medium was enriched to maintain similar delivery of glucose and amino acids. The culture behavior at CSPR 20 and CSPR 40 was comparable, however, in CSPR 10 the growth rate slowed and the cell size increased, while the cell specific mAb productivity and the high viability were maintained. The nutrient uptake rates were slightly reduced, indicating a more efficient mAb expression, and a metabolism shift. To understand the mechanisms underlying this phenomenon, we analyzed the gene expression changes in a framework based on a mechanistic model of the main metabolic pathways. For this approach, mRNA sequencing was performed and the gene expression changes were mapped to individual reactions within relevant metabolic pathways. The gene expression was clearly distinct in CSPR 10 compared to CSPR 20 and 40, confirming the metabolism shift. The low CSPR led to downregulation of pentose phosphate pathway and TCA cycle, upregulation of the lipid metabolism, reactive oxygen species, and changes in the glycolysis and amino acid metabolism. The N-glycosylation was unchanged but a shift in the charge variant was observed. Charge variant shift seemed to be due to the longer residence time of the mAb in the bioreactor at low CSPR, and resulting from deamidation of asparagine 30 on the light chain. The gene expression revealed that methionine oxidation could also occur and contribute to the charge variant shift.