CHARACTERIZING THE EFFECT OF GLUTAMINE SUPPLEMENTATION ON ASPARAGINE AND GLUTAMINE METABOLISM USING ^{13}C METABOLIC FLUX ANALYSIS

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Upstream development efforts often focus on improved productivity. Among those efforts, improvements in medium formulations have translated into greater titers. To continue this historical trend, a better understanding of the cell metabolism is warranted for guiding efficient utilization of medium components to improve titer while minimizing byproducts. ^{13}C Metabolic Flux Analysis (13C MFA) offers opportunities to study metabolic phenotypes by applying isotope tracers to estimate the intracellular fluxes through metabolic pathways.

In this work, ^{13}C MFA was applied to study the effects of glutamine supplementation by ^{13}C parallel labelling of cultures with [U-^{13}C]asparagine, [U-^{13}C]glutamine and an a mixture of [U-^{13}C]glucose with [1,2-^{13}C]glucose. The study was focused on two metabolic states characterized by glutamine consumption in the early exponential phase and glutamine production in the late exponential phase of a fed-batch culture. To quantify individual metabolic pathway activity, metabolic flux maps were generated for the glutamine supplemented feeds compared to a control case with glutamine in the initial medium. The glutamine supplementation condition resulted in redistribution of the fluxes in the TCA cycle. Furthermore, measurements of the enrichment of cell protein indicate different allocations of the fed nutrients into generated biomass for the glutamine supplemented condition. Comparison between the early and the late exponential phases provided novel insights on how glutamine modulates CHO central carbon metabolism and supports the important role of glutamine as a major source of energy for cell proliferation. These findings contribute towards an improved characterization of the metabolism of industrial cells with useful implications for optimizing medium and feed development.