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Systems Analysis of CHO cell metabolism for enhanced fed-batch process performance: Identification of novel growth inhibitors and their control

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SYSTEMS ANALYSIS OF CHO CELL METABOLISM FOR ENHANCED FED-BATCH PROCESS PERFORMANCE: IDENTIFICATION OF NOVEL GROWTH INHIBITORS AND THEIR CONTROL BY TWEAKING AMINO ACID METABOLISM

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Over the past decade, process development efforts have led to attaining titers close to 3-4g/L on a routine basis in CHO fed-batch cultures. However, with the advent of biosimilars and the ever expanding therapeutic portfolio of innovator drugs, there is a growing need for enhancing the throughput of fed-batch cultures in order to meet the product requirements with the limited manufacturing capacity. The classical hurdle for higher productivities in fed-batch cultures is the sub-optimal metabolism of mammalian cells that causes accumulation of byproducts, including lactate and ammonia, resulting in growth inhibition. With a robust CHO expression system, our group has previously demonstrated the use of a pH-linked culture glucose control strategy called HiPDOG, which efficiently suppresses the production of lactate and ammonia, enabling higher cell densities and productivities. Even under such conditions with significantly reduced accumulations of conventional inhibitors, cell growth eventually halts.

Systems biology approach was employed to these near-optimal fed-batch cultures to probe and determine various biochemical and biophysical factors that could be responsible for growth inhibition. Metabolomics analysis of CHO fed-batch cultures and add-back experiments with purified compounds led to the identification of a number of metabolites as novel growth inhibitors. A significant fraction of these inhibitors are intermediates or byproducts of cellular catabolism. Biosynthesis and accumulation of these inhibitors was determined to be due to high levels of certain medium components. Controlling the levels of these components at lower concentrations in fed-batch cultures led to reduced accumulations of the inhibitors, resulting in higher peak cell densities and titers. Details of the novel inhibitors, their nutrient source and their effect on cell physiology, along with strategies employed to automatically control accumulations of these inhibitors in fed-batch processes will be discussed.

Figure 1 – Arial 10 pt Italics