DESIGN OF EXPERIMENTS GUIDED CONTROL OF WASTE INHIBITORY BY-PRODUCTS IN HIGH DENSITY CHO CELL CULTURES

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Chinese Hamster Ovary (CHO) cells in high cell density cultures consume large amounts of nutrients for biomass synthesis and protein production. Inefficient utilization of these supplemented resources due to sub-par metabolic networks in CHO cells leads to accumulation of pathway intermediates and by-products that negatively impact the cell culture performance. Previously, we have identified multiple inhibitory by-products during a fed-batch shake flask process that were shown to negatively impact cell growth and titer yields. These inhibitory by-products were found to be a part of amino acid and central carbon metabolism pathways of CHO cells eventually leading to reductions in cell performance. In this study, we demonstrate the control of these inhibitory by-products with the help of strategic cell culture medium construction using a two-level Design of Experiments (DOE).

First, a pathway analysis guided by KEGG database and extensive literature review was conducted to identify the precursors that lead to the accumulation of the different inhibitory by-products. This produced a matrix of amino acid precursors which were verified for their contribution to the inhibitory by-product accumulation using an over-supplementation study. Once confirmed, the final precursor matrix was input to a two-level DOE where at the first level, a screening design led to reduction of the design space from 10 amino acids to 5 to select for the significant contributors of inhibitory by-product buildup. At the second level of DOE, the optimal concentration determination of each of the 5 significantly contributing amino acids was performed using a response surface design. The resulting reduced amino acid concentrations were validated in both batch and fed-batch cultures to yield better growth and productivity conditions. Overall, a 15% and 55% improvement in peak viable cell densities (VCDs) and 7% and 50% enhancement in IgG production was observed in batch and fed-batch process respectively for cells cultured in the DOE-guided optimized medium as compared to the control medium.

This study successfully showed the utility of complex statistical design of experiment methodologies in the medium formulation keeping the metabolism of CHO cells in scope. The strategy applied in this study significantly improves CHO cell culture performance solely by controlling multiple nutrient factors that lead to accumulation of toxic inhibitory by-products. Implementing such mathematical techniques in cell culture can lead to the generation of better production processes to meet the high demand for therapeutic protein production now and in the future.

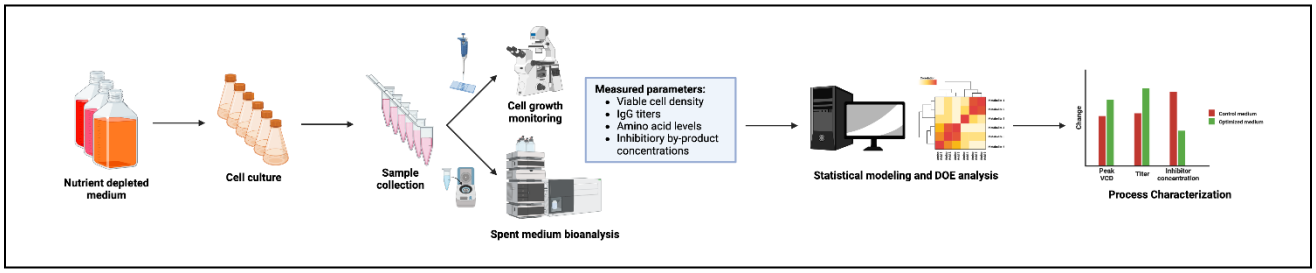

Figure 1 – Inhibitory by-product control workflow using medium optimization by statistical DOE approach

Reference: Kuang, B., Dhara, V.G., Hoang, D., Jenkins, J., Ladiwala, P., Tan, Y., Shaffer, S.A., Galbraith, S.C., Betenbaugh, M.J. and Yoon, S., 2021. Identification of novel inhibitory metabolites and impact verification on growth and protein synthesis in mammalian cells. Metabolic engineering communications, 13, p.e00182.