

SHORT CHAIN FATTY ACIDS PRODUCED BY CHO CELLS ENHANCE THEIR SPECIFIC PRODUCTIVITY IN FED-BATCH CULTURES

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Chinese hamster ovary (CHO) cells are workhorses for recombinant therapeutic protein production. In fed-batch processes, CHO cells produce several metabolic byproducts derived from amino acid catabolism. A fraction of them accumulate to concentrations that can cause growth inhibition in culture¹. Suppressing the levels of these byproducts either by controlling the supply of amino acids in cell culture or by genetically modifying the biochemical pathways producing these byproducts significantly enhances cell growth and productivity. Interestingly, some of these byproducts can have physiological roles beyond growth inhibition. For example, some of these byproducts are short chain fatty acids (SCFAs) including butyrate, isobutyrate and isovalerate, which have been shown to act as HDAC inhibitors². Butyrate is a known specific productivity (q_P) enhancer. The q_P enhancing effect of isovalerate and isobutyrate is not fully established yet. Further, the dynamics in production of these byproducts and the impact of these byproducts on fed-batch culture productivities is yet to be ascertained.

Isovalerate and isobutyrate have been previously shown to be byproducts of leucine and valine catabolism^{1,3}, respectively. In the current study, first, the source of butyrate production was ascertained. Limiting the concentration of one or more branched chain amino acids (BCAAs) resulted in reduced butyrate production suggesting that BCAA catabolism plays a role in butyrate production. Next, the q_P enhancing role of isovalerate and isobutyrate on CHO cells was probed. Suppression of SCFA production in CHO cell fed-batch cultures either by knockout of the BCAT1 gene or by a restricted supply of amino acids resulted in a drop in the q_P . Whereas supplementation of SCFAs back to BCAT1 KO or amino acid limited cultures restored the q_P back to control culture levels. This suggested that isovalerate and isobutyrate can act as q_P enhancers⁴.

Subsequently, the dynamics of SCFA production in CHO cell fed-batch cultures were investigated. Production rates of SCFAs were observed to be negatively correlated with the glucose consumption and lactate production rates in CHO cell fed-batch cultures. For example, across multiple cell lines, reducing lactate production by maintaining glucose at very low levels by using high-end pH-controlled delivery of glucose (HiPDOG) technology significantly enhanced SCFA accumulation compared to non-glucose-limiting fed-batch cultures. HiPDOG cultures had higher q_P and yielded higher titers. Further, processes that metabolically shift to consume lactate produced more SCFA compared to processes that didn't consume lactate. This could be one of the reasons why shifted cultures have better q_P and productivity than non-shifted cultures. Understanding of the additional roles of newly identified metabolic byproducts of CHO cells can help contrive strategies for developing more robust and high titer processes.

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