Engineering Conferences International
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Cell Culture Engineering XV | Proceedings

Spring 5-10-2016

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IMPROVING THE METABOLIC EFFICIENCY OF MAMMALIAN CELLS AND ITS IMPACT ON GLYCOPROTEINS QUALITY

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Key Words: HEK293, Fed-batch, protein glycosylation, PYC-overexpression.

Glycosylation is a critical quality attribute for recombinant therapeutic proteins, which can be impacted by a number of process conditions, including waste metabolite accumulation. While fed-batch strategies that consist in substituting or controlling the main substrates at low concentrations have proven generally effective at improving protein titer, they can also adversely affect product glycosylation. Metabolic engineering strategies aiming at reducing by-product formation may thus be beneficial for ensuring product quality consistency. In this work, we have specifically investigated the impact of PYC2 overexpression on the quality of a recombinant glycoprotein of therapeutic interest, the interferon α2b (IFNα2b) that has one O-glycosylation site. To this end, batch and fed-batch cultures were performed and product characteristics were measured for both the PYC expressing HEK293 clone and the parental cells.
SDS-PAGE and Western Blot analysis of batch culture harvests revealed two distinct bands corresponding to glycosylated and non-glycosylated fractions of IFNα2b, as subsequently confirmed via SDS-PAGE analysis of purified samples loaded along with a non-glycosylated commercial standard produced in *E.coli*. As inferred from densitometry analysis of the gels, the cultures with PYC-expressing cells were shown to sustain a significantly higher percentage of glycosylated IFNα2b at the late stage of the culture, which was correlated with the prolonged viability and reduced accumulation of waste metabolites. Differences between the two cell lines in terms of cell viability and protein quality were even more pronounced when performing fed-batch cultures during which glucose was maintained at high levels. To investigate the potential impact of ammonia, batch cultures with various glutamine substitutes were also performed. Among the different substitutes tested, pyruvate led to the lowest ammonia production with no significant impact on protein titer. Of salient interest, the results suggest that substituting glutamine by α-ketoglutarate, glutamate or pyruvate may allow to maintain a higher fraction of glycosylated proteins during late-stage batch cultures.