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# Optimization of glycosylation and charge distribution through culture parameters and supplements

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## OPTIMIZATION OF GLYCOSYLATION AND CHARGE DISTRIBUTION THROUGH CULTURE PARAMETERS AND SUPPLEMENTS

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Culture parameters are known to have significant impacts on product quality, although these effects are sometimes cell-line dependent and the directionality of the effect has to be determined empirically. We will present data from three case studies where glycosylation or charge distribution was modified to match the reference molecule or to reduce variability. In the first case study, glycan optimization for a biosimilar will be described. Galactose, fucose, and mannose levels were optimized through screening of raw materials and process parameters. A range of media, feed, alternate sugars, metals and other supplements as well as temperature set points were tested. Specific conditions were able to change galactosylation by 50 – 70%. Impact of a fucose analog in culture was also studied. Temperature shift had a significant impact on glycan and charge distributions. In the second case study, the strategy for achieving consistent charge distribution for a cell line with variable growth and lactate profiles will be discussed. When lactate was consumed, the acidic species amount was found to be relatively high, whereas, when lactate was accumulating, the acidic species level was low. In the third case study, impact of  $\text{Cu}^{2+}$  on charge distribution will be discussed. The cell line had initially shown varied productivity, which was traced back to copper deficiency. Supplementation of  $\text{Cu}^{2+}$  however, led to change in charge distribution, and a titration study was carried out to identify the ideal  $\text{Cu}^{2+}$  level. Through these experiences, we have established a tool box that can be used to achieve desired product quality effectively and efficiently.