TRANSCRIPTIONAL AND METABOLIC RESPONSE OF CHO CELLS TO DIFFERENT CARBON DIOXIDE CONCENTRATIONS

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A critical process parameter that influences cell culture behavior is the dissolved carbon dioxide (dCO_2), however information on its effects is limited compared to other variables more commonly studied. It is known that in Chinese Hamster Ovary (CHO) cell cultures, dCO_2 can affect cell growth and viability, protein productivity and critical quality attributes of the product such as glycosylation pattern and the charge variants profiles, but little is known on its effect on transcription of key genes. In this work three different conditions of CO_2 in the gas phase of shake flasks were evaluated (0, 5 and 20%) to determine their impact on cultures of CHO cells producing a recombinant monoclonal antibody (mAb). The transcription level of six key genes (lactate dehydrogenase (*ldhA*), glutaminase (*gls*), carboxipeptidase D (*cpD*), carbonic anhydrase (*ca5B*), IgG Light Chain and IgG Heavy Chain) related to CHO metabolism and mAb production was determined at two different culture times (48 and 120 h) for all CO_2 conditions. No changes in the level expression for *cpD* was observed in any of the conditions, but changes for *ca5B* were observed in cultures at 0% CO_2. A thorough characterization was performed of kinetic and stoichiometric variables, as well as productivity, and quality of the MAb produced. The cultures under a 20% CO_2 atmosphere showed the highest MAb production in comparison to controls cultures (5% of CO_2), whereas cultures at 0% CO_2 had the lowest cell growth and productivity. At the metabolic level, cultures at 5 and 20% CO_2 had a high glucose consumption rate and a marked metabolic switch of lactate production to consumption when L-glutamine was depleted. In contrast, cultures at 0% CO_2, showed the lowest glucose consumption rate but accumulated lactate at the highest concentration. Differences in the glycosylation pattern and charge variants profiles were observed. Compared to control cultures, a greater relative abundance of both acid and basic species was observed for cultures under 0% CO_2, whereas at 20% CO_2 a lower relative abundance of both species was observed. There was a greater relative abundance of galactosylated glycans in cultures at 0% CO_2, whereas there was a significant increase in relative abundance for high mannose and hybrids glycans for the cultures at 20% CO_2. The results of this work show that CO_2 impacts, not only CHO cell metabolism and quality and quantity of the MAb produced, but also the transcription of selected genes.

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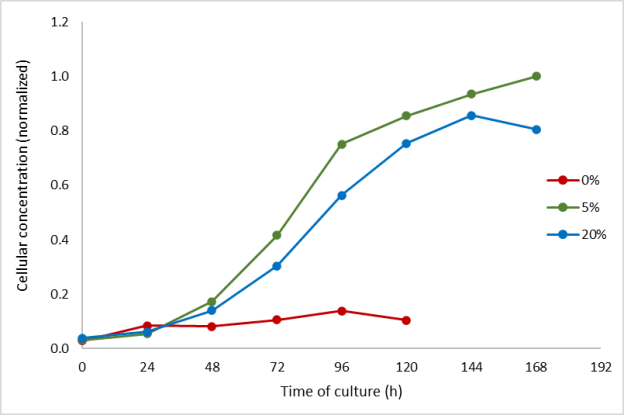

Figure 1. Cellular concentration at different levels of CO_2.

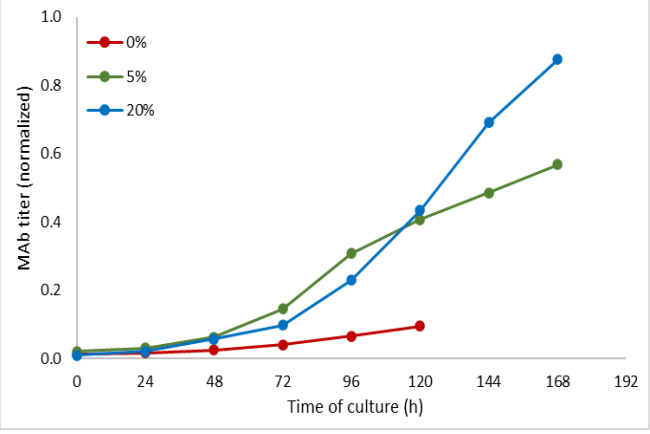

Figure 1. MAb concentration at different levels of CO_2.