# APPLICATION OF $^{13}C$ FLUX ANALYSIS TO DETERMINE IMPACTS OF MEDIA ALTERATIONS ON INDUSTRIAL CHO CELL METABOLISM

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Industrial bioprocesses place extraordinary demands on the metabolism of host cells to meet the biosynthetic requirements for maximal growth and protein production. Identifying host cell metabolic phenotypes that promote high recombinant protein titer is a major goal of the biotech industry. $^{13}C$ metabolic flux analysis (MFA) provides a rigorous approach to quantify these metabolic phenotypes by applying stable isotope tracers to map the flow of carbon through intracellular metabolic pathways. We have conducted a series of $^{13}C$ MFA studies to examine the metabolic impacts of altering the composition of a proprietary chemically defined growth medium on CHO cell metabolism.

CHO cell cultures characteristically produce excess ammonia and lactate as byproducts, both of which are toxic at high concentrations. Whereas lactate is often consumed during stationary growth phase in CHO cell cultures, ammonia continues to accumulate in the extracellular media throughout the course of cell growth due mainly to glutamine catabolism. For CHO cells that utilize glutamine, rational media design can alleviate ammonia stress from the cell culture. However, manipulating carbon sources in the growth medium can also have negative effects on cellular metabolism such as decreased culture growth, viability, recombinant protein productivity, or longevity. This study highlights a rationally engineered cell culture medium that successfully reduces culture ammonia levels by 40% while maintaining the original metabolic phenotype.

First, the basal media developed in-house by Sanofi was chemically altered to cause CHO cells to produce significantly less ammonia byproduct. This low ammonia-producing media variant was experimentally developed by altering the ratio of carbon sources in the media to strategically reduce flux through metabolic pathways that result in ammonia production while supplementing complementary, non-ammonia producing pathways to balance metabolism. This altered media variant successfully decreased the ammonia concentration in industrial CHO while maintaining culture growth, viability, and specific productivity.

Parallel $^{13}C$ MFA studies were performed on IgG-producing CHO cells grown identically in three media variants: the basal control media, the low-ammonia media, and the low-ammonia media supplemented with basal ammonia levels. The latter media was used to control for any direct effects of changing ammonia concentrations on cellular metabolism. $^{13}C$ labeling studies utilizing [U-$^{13}C_5$]glutamine and [1,2$^{13}C_2$]glucose were carried out in parallel for each condition. From the comparison of the $^{13}C$ flux analysis across the three media types, we have concluded that the media alterations did not have a significant impact on the intracellular metabolism of CHO cultures. This suggests that Sanofi can use their newly developed media formulation to decrease toxic ammonia buildup in IgG-producing CHO cell lines without significantly altering host metabolic phenotype or productivity.