Stable Isotopic Labeling of 1,2$^{13}C_2$-Glucose and 1,6$^{13}C_2$-Glucose for Tracing CHO Cell Metabolism and Mass Spectrometry-based Metabolic Flux Analysis

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Abstract

Approximately 80% of therapeutic monoclonal antibodies are produced by Chinese Hamster Ovary (CHO) cell lines which have been optimized in studies involving nutrient profiling and genomic modification to maximize performance in culture. However, changes in metabolites between culture states and how they are related to product quality and quantity have not been well characterized. CHO cell lines can rapidly proliferate utilizing glucose as a main source of energy. This metabolic state is known as the Warburg effect and leads to high levels of lactate production by the cell. The high accumulation of lactate and cellular debris in culture can hinder cell growth in upstream production and negatively impact downstream protein purification by clogging filters. Previously, our team achieved high protein yield through a design-of-experiment (DoE) approach by studying feed strategies and process parameters, but a fundamental understanding of the inner metabolic state of the cell could lead to more accurate predictions on how the desired cell state can be achieved and maintained. Therefore, it is essential to trace the distribution and flux of downstream metabolites. This project proposes a model study using stable isotopes 1,2 ^{13}C2-Glucose and 1,6 ^{13}C2-Glucose to trace our in-house VRC01 cell line for metabolic flux analysis (MFA) by utilizing LC-MS. This linkage of intracellular data with product quality information may be used to understand and develop potential intracellular control strategies to improve and maintain product quality attributes of monoclonal antibodies during commercial manufacturing.

Introduction

Chinese Hamster Ovary (CHO) cell lines are among the most common host for producing therapeutic monoclonal antibodies [1, 2]. However, the Warburg effect of CHO cells illustrates its inefficient metabolism. Nutrients such as glucose are not exclusively used to produce recombinant proteins or biomass. Based on publications, up to 70% of glucose could be turned into waste product, which can negatively impact cell growth or culture performance [4]. The use of stable isotopic labeled glucose enables the examination of intracellular metabolism and the changes in metabolite levels from a pathway-centric perspective [5, 6]. This poster showcases the cell culture results from an example run where a temperature shift significantly slows down the cell metabolism, determined by evaluating the cell uptake rate of glucose.

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Methods

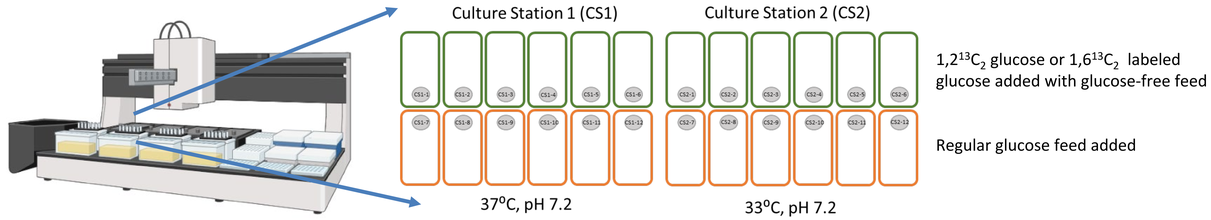

Figure 1. Ambr 15 microbioreactor experimental setup. Both culture station 1 and culture station 2 were started and set at the same conditions at the beginning of this experiment. The temperature shift happened at the same time where labeled glucose feeds were introduced to the culture to ensure the temperature shift captures the change in cell metabolism. Each condition has 6 biological replicates (N=6).

Results

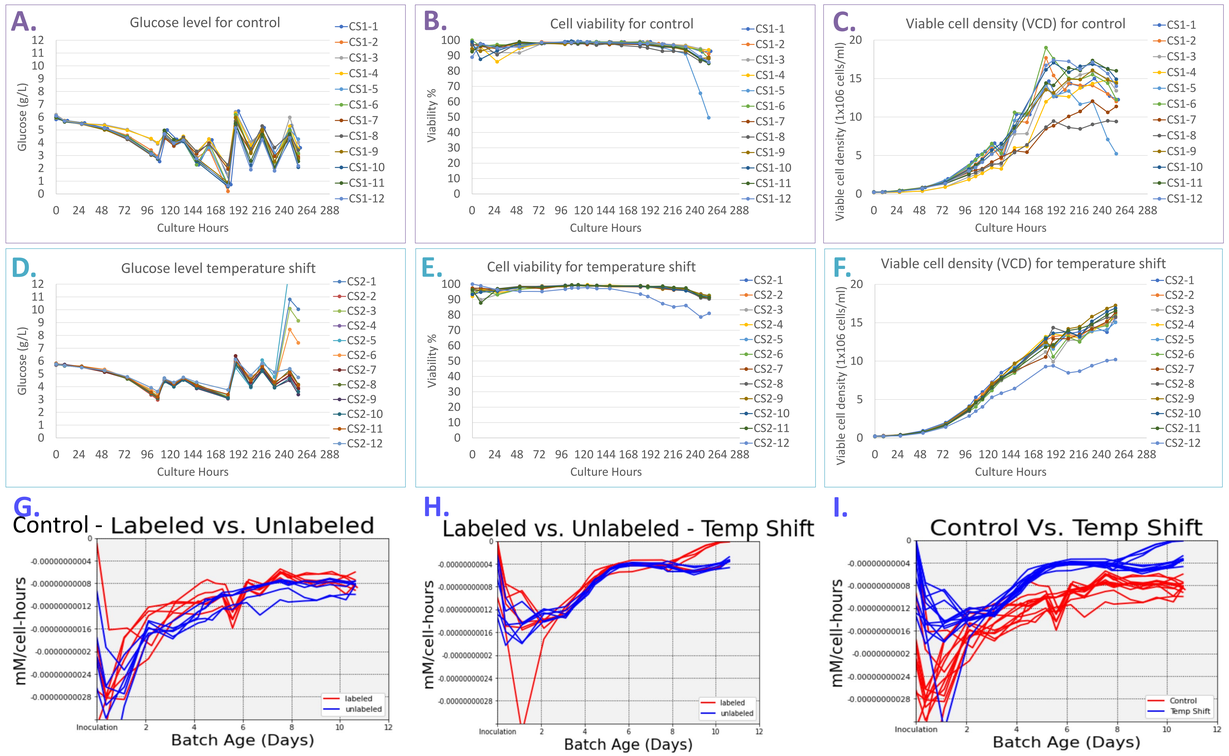

Figure 2. Cell culture results from Ambr 15 microbioreactors. Graphs B and E indicated cell viability remained above 80% during this run. Graphs A and D captured the temperature shift impacting the cell metabolism, where the cells behaved similarly during the first 3 days, but after temperature shifted around 100 culture hours the cells' glucose consumption slowed down. Graph I is a combined smoothing algorithm of all biological replicates for control and temperature-shifted groups; at culture age day 6, it was clear that temperature shift significantly impacted the glucose consumption rate.

Discussion

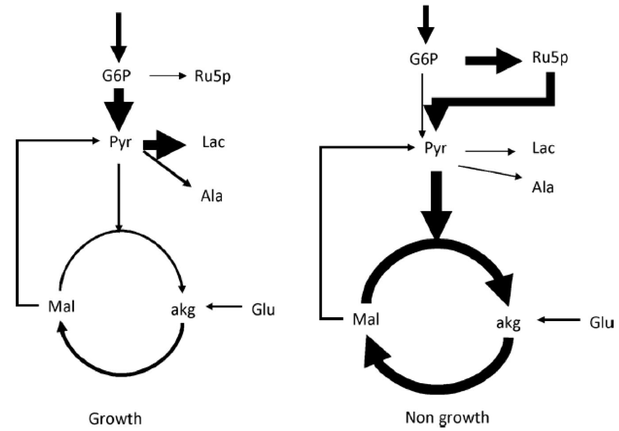

Figure 4 [9]. An example of CHO cell metabolic pathway (pentose phosphate pathway) shows a difference in flux pattern between cell growth and nongrowth phases.

- Stable isotopes 1,2 ^{13}C2-Glucose and 1,6 ^{13}C2-Glucose are chosen from a tracer selection study by Crown et al. They concluded that those two tracers give the most abundant metabolite coverages compares to the other 17 tracers in the study. Their statistical modeling also shows that the combination of 1,2 ^{13}C2-Glucose and 1,6 ^{13}C2-Glucose works synergistically by 20-fold more metabolites analyzed than if each tracer looked at individually. The ^{13}C will allow us to trace the metabolites through the metabolic pathways, and the rate at which they convert (change between timepoints) will determine the flux.
- Ambr 15 microbioreactor system allows the parallel labeling experiment run in 6 replicates for each group; off-line monitoring of cell nutrient consumption or waste produced in each vessel are measured by Flex 2 everyday during the culture run.
- Metabolic flux analysis (MFA) uses in vivo isotopic labeling patterns of downstream intracellular metabolites coupled with mathematical modeling to quantify biochemical reaction rates through the major metabolic pathways such as the TCA cycle, Pentose Phosphate pathway, and glycolysis.

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