Editorial

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Nutrient and metabolite analysis: understanding cell culture

Today's challenges are associated with the need to achieve high levels of productivity to reduce operational cost while maintaining acceptable and consistent product quality to ensure safety and efficacy of the drugs produced.

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Fed-batch cultures of mammalian cells are currently the most common platform for the synthesis of biopharmaceutical proteins. While fundamental challenges associated with establishing this as a robust platform have been solved, today's challenges are associated with the need to achieve high levels of productivity to reduce operational cost while maintaining acceptable and consistent product quality to ensure safety and efficacy of the drugs produced. Accurate metabolite analysis is critical to understanding both the intracellular and extracellular environments in cell culture, which could lead to greater understanding of the process impacts on productivity and product quality. Cell growth is associated with the consumption of the carbon source, amino acids, vitamins and other essential nutrients and the production of by-products such as lactate and ammonium. The concentration profile of almost all these nutrients during the cell culture process could potentially impact productivity or product quality. Furthermore, today's technologies also enable the quantitation of many of the intermediate metabolites consumed and produced in intracellular reactions, which could potentially shed more light on the metabolic state of the cell.

Glucose is almost universally used as the primary carbon source for most industrial mammalian cell culture processes. Accurately measuring glucose concentration is critical to prevent its depletion and accompanying loss of cell viability and process performance. Glucose levels could also impact the levels of glycation, a covalent modification of a protein at lysine residues, in certain monoclonal antibodies. Hence, it is critical to control its concentration if glycation is to be constrained below certain levels [1]. Mammalian cell growth is usually accompanied by lactate synthesis due to the Warburg effect [2]. While the intrinsic effect of lactate on cell growth and productivity is a matter of debate, increased lactate synthesis is usually accompanied by excessive base addition for pH control, leading to increased osmolality and altered productivity. Similarly, ammonium is a byproduct of amino acid catabolism, and elevated ammonium levels might impact the glycosylation profile [3] and/or could impact productivity. [4] Hence, controlling lactate and ammonia are common goals in cell culture process development. Glucose, lactate and ammonium are usually measured using commercially available multi-functional analyzers [5], and are usually measured using daily off-line samples during both development and commercial/clinical production batches.

Measuring the concentration of amino acids could be critical from a variety of perspectives, and standard HPLC-based assays are available for this purpose [6]. Amino acids are usually measured using off-line samples during development. Since the depletion of certain amino acids could correlate with decreased growth and/or specific productivity, amino acid

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concentrations in the culture need to be monitored during basal and feed medium development. Recently, it was reported that tyrosine depletion could also correlate with the presence of certain sequence variants involving the replacement of tyrosine with phenylalanine or histidine [7]. In addition, increased concentration of certain amino acids, such as asparagine or glutamine, could lead to elevated concentration of ammonium ions, which might then impact productivity or product quality.

It has long been known that vitamins are essential for cell growth [8]. Recently, the concentration of B vitamins has been shown to impact the color of the drug substance [9,10]. Hence, it is critical to ensure vitamins are not depleted, while also not added in excess. To accomplish this, vitamin concentrations are usually measured during development of the culture medium and process using standard HPLC-based methods [11].

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Several recent publications have described the effects media components present in trace quantities on productivity or product quality. The concentration of copper has been linked to the presence of free thiol and proline amidation [12,13], as well as to the control of lactate metabolism [14]. Iron source and concentration have been shown to impact the color of the final drug substance as well as the charge variant profile [9]. Relative concentration of zinc and copper has been shown to impact C-terminal lysine [15]. The concentration of most of these trace nutrients are usually not monitored during clinical or commercial production unless such investigation is necessitated by unexpected process performance or execution. However, monitoring and optimizing their levels is important during media and process development. Trace metals are usually assayed using inductively coupled plasma–mass spectrometry-based assays. If these metabolites cannot be readily assayed, the effect of a dose-dependent addition to the culture medium needs to be studied for optimizing their concentration.

In addition to the standard metabolite analyses that are currently performed, development of systems biology has provided the cell culture engineer with additional tools to investigate cellular metabolism. Of these tools, there has been increased interest recently in the use of mass spectrometry-based metabolomics tools since it enables quantitation of intracellular small-molecule metabolites, which allows investigation of the physiology of the cell more directly than transcriptomic or proteomic tools [16,17]. Metabolomic approaches, along with other 'omic' approaches, continue to enhance our knowledge of metabolism, which should help design better process in the long run. While it is possible to envision a future when these tools are widely used during development, it is not currently clear if the benefits derived from the use of these tools for the time-constrained development of cell culture processes justify the cost and effort.

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