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Current Opinion in Chemical Engineering

Advances in process control strategies for mammalian fed-batch cultures

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Current Opinion in Chemical Engineering 2018, **22**:34–41

This review comes from a themed issue on **Biotechnology and bioprocess engineering: biomanufacturing**

Edited by **Michael Betenbaugh** and **Nigel Titchener-Hooker**

https://doi.org/10.1016/j.coche.2018.09.002

Introduction

The current pipeline of new protein therapeutics has expanded vastly to treat a wide array of diseases including cancer, autoimmune diseases, and neurodegenerative disorders. Mammalian cell culture has been the primary manufacturing host for the production of glycosylated antibodies, recombinant enzymes, and anti-hemophilic factors. Batch, fed-batch, and perfusion have been the standard operating platforms since the first biologic manufacturing was established over 30 years ago.

Considerations in choosing between fed-batch and perfusion mode of operation include economic reasons [1,2•], protein stability, and manufacturer's existing equipment capability. In the past 5 years, concentrated fed-batch [3•] and a new hybrid model of using perfusion in the inoculum followed by high density fed-batch process have been adopted [4,5]. Along with these new modes of operation, PAT approaches have been developed for process monitoring and encouraged by regulatory agencies. Beyond the typical temperature, pH, and dissolved oxygen (DO) sensors critical for controlling bioprocesses, these newer sensors can probe in-depth cell physiological state through continuous monitoring, and/or guide real-time feedback control. The focus of this review is on recent trends in fed-batch feed media design and feed control strategies enabled by these advanced sensors to deliver higher productivities and more robust protein product quality.

Process improvement through feed design

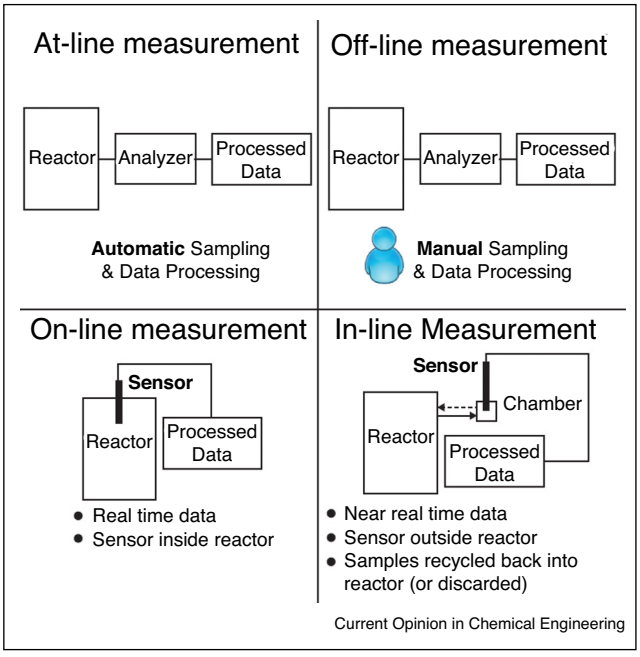
Recent feed media design emphasizes a rational or quantitative approach [6]. This approach involves calculating the specific consumption of substrates such as amino acids or glucose, and designing feed media to supplement only depleted nutrients in a quantitative manner. Feed rebalancing can be performed by choosing a surrogate substrate as an indicator of cell metabolism and normalizing all other carbon sources and amino acids to this surrogate by the ratio of their consumption rates [7]. One benefit of this strategy is to prevent overfeeding certain nutrients, thereby avoiding the production of inhibitory metabolites for cell growth or protein production [7,8••].

The advent of high cell density seeding processes requires the development of highly concentrated feed media, increasing concerns around feed media stability due to precipitation or degradation of certain nutrient components during storage. Recent studies have elucidated certain feed components that can be included or substituted to address this issue. Bicarbonate can lead to increased rates of glutamine degradation, and pyruvate improved the stability of concentrated feed [9]. Surfactants including polysorbate 80 (PS80), PS20, or poloxamer 188 have also been reported to improve the solubility and stability of enriched feed media [10]. Addition of antioxidants such as thiazolidine may help to improve the stability of redox sensitive media components [11•]. Finally, replacement of tyrosine or cysteine with their derivatives such as phosphotyrosine [12] or s-sulfocysteine [13•], respectively, may increase tyrosine solubility or reduce cysteine degradation, thereby improving the stability of concentrated feed media. Proper storage of media is also important as photo-degradation of components giving rise to radical species can be detected in light-exposed media [14].

Overview of process monitoring and controller systems

Bioprocess monitoring can be classified as off-line, at-line, in-line or online monitoring according to the placement of the sensor or analyzer instrument relative to the bioreactor, and the delay between sample withdrawal and analysis on the instrument (Figure 1). In the recent five years, the application of Raman and dielectric spectroscopy in fed-batch cultures has gained prominence due to the ability to detect several analytes simultaneously and monitor cell physiology in real time.

Figure 1



Classification of in-line, on-line, at-line and off-line measurement of bioreactor parameters.

Raman and dielectric spectroscopy

Raman monitoring of mammalian cell bioprocesses has grown increasingly popular since 2011, when Abu-Absi *et al.* published the ability to monitor glutamine, glutamate, glucose, lactate, ammonium, viable cell density (VCD), and total cell density (TCD) in a bioreactor with an antibody-producing CHO cell line [15]. An important advantage of Raman technology is the large amount of information that can be obtained from a single probe: in addition to the aforementioned metabolites and cell counts, models have been reported for in line prediction of osmolality [16], antibody titer [17•], antibody glycosylation [18], and amino acid concentrations [19•]. Raman limits of detection vary based on the experimental setup (e.g. laser source, collection times) as well as interference from the medium in which the analyte is located; however, accurate predictions in the millimolar concentration range in cell culture media are commonly reported. For example, Berry *et al.* reported calibrations over ranges of ~3–14 g/L glucose (~17–78 mM), ~0.5–2.5 g/L lactate (~6–28 mM), and ~2–5 mM glutamate [20].

Analysis of Raman data requires the collection of a set of spectra and corresponding accurate offline measurements. Multivariate statistical techniques, such as partial least squares (PLS) regression, are used to link spectral data with model-predicted outputs. Useful models can be built with as few as 45 measurements and 34 calculated values [15]. However, prediction accuracy is greatest when the predicted data lie within the ranges established in the calibration dataset, and generalizing Raman models to different cell lines and processes can require larger datasets for calibration (Table 1).

Care must be used when developing and applying chemometric models. Raman models may detect components that correlate with the predicted component rather than directly sensing the predicted component. If the correlation between the detected analytes and the predicted analyte is altered, the model will fail to predict accurately; thus, there is risk in using and applying such models. Calibration sets should include a range of concentrations of predicted and interfering components that is representative of the various conditions for which the calibration set will be applied. In depth discussions of chemometric modeling can be found in several reviews and texts [21].

Raman models that can be uniformly applied for model prediction at 3-L, 200-L and 2000-L bioreactor scales have been evaluated [20]. Calibration models for glucose, lactate, and osmolality in small-scale (3-L) bioreactors were able to predict metabolite concentrations in a 2000-L bioreactor with less than 10% RMSEP (root mean square error of prediction), relative to the maximum measured process value. However, in order to accurately predict cell densities, glutamate and ammonium at the 2000-L bioreactor scale, at-scale data needed to be included in the calibration model.

Dielectric or impedance spectroscopy estimates viable cell biomass by applying alternating electric current at various frequencies and measuring the capacitance. The capacitance is correlated to viable cell biomass due to the ability of intact cell membranes to hold electrical charge. Linear correlation between capacitance and viable cell biomass can be used in the growth phase of a fed-batch or batch process, however multivariate correlation methods such as PLS (partial least squares) are better-suited to describe the changing properties of cell-size, permittivity, and cell biovolumes in the stationary and decline phase [22,23]. Capacitance measurements have been widely applied in mammalian cell culture to detect apoptotic events [24], determine feeding amounts [7], and monitor stem cell differentiation [25].

Feed control mechanisms

Process control algorithms

Bioprocesses employ similar control strategies as used in other chemical industries (Figure 2). Most of the existing bioprocess control loops are handled by conventional PID (proportional, integral and derivative) feedback controllers, for example bioreactor pH, DO and glucose control.

Non-linear model-based control applies a mathematical model of substrate consumption, metabolite production,