Benefits of Using Real-Time In-Process Data in Mammalian Cell Culture

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The manufacturing systems used for production of mammalian cell-based protein therapeutics have the capability to provide real-time data for a number of cell culture parameters, including pH, DO, and temperature. These data can be used in statistical analyses to help define a process's control strategy or to monitor the process as part of continuous process verification (CPV); occasionally they are used to investigate a process deviation or failure. However, some manufacturers do not use this data to their full advantage, resulting in opportunity costs on the manufacturing floor. Specifically, better use of the tools available to monitor process performance during a production run, particularly when combined with proper training of manufacturing personnel, provides significant opportunities for both improving processes during development as well as averting poor process performance and even potentially batch losses during routine manufacture.

The BioProcess Technology Group (BPTG) within BDO, which has experience assisting clients to improve recombinant protein manufacturing processes, has noted a number of instances where timely review of real-time data by trained operators would have increased the likelihood of batch success, or at least mitigated the impact of batch failure, if the available real-time data were reviewed and understood. Three such examples are described below.

- During a typical 14-day CHO cell culture process, bioburden samples were collected prior to harvest and submitted to QC for evaluation. Five days after the culture was harvested and processed through costly depth filters and two chromatography columns, the bioburden results were reported as "too numerous to count". During the subsequent investigation, in which the gassing trends were reviewed, a dramatic increase in oxygen demand starting halfway through Day 13 was noted. Although this batch could ultimately not be salvaged, the costs associated with the recovery and initial purification steps could have been avoided if the gassing trends had been reviewed in a timely manner (i.e., prior to culture harvest) by an operator trained to understand the culture conditions that might lead to significant increases in oxygen demand.
- As part of a process scale-up, an at-scale engineering run was performed in which the culture harvest failed when the second stage filters clogged, resulting in only 20% of the available cell culture fluid being processed. Given that this was the first run at the proposed clinical manufacturing scale, no prior data at this scale were available, meaning that at-scale trend analyses could not be performed. However, data were available from more than 16 development runs that were used to define the process control strategy. A review of these data highlighted that all of the development runs achieved maximum viable cell densities approximately two-fold higher than the engineering run and were harvested with viabilities above 70%, whereas the engineering run had a viability below 50% at harvest, which likely led to the clogged filters.

An investigation into the engineering run revealed a flawed gassing strategy. Briefly, oxygen was being supplied through a drilled hole sparger with a 100-standard liters per minute (SPLM) mass flow controller (MFC). No cap was placed on the MFC and a microsparger was not used for peak oxygen demand. The gassing profiles of the engineering run revealed that the oxygen MFC was over 50 SLPM from Day 3 onward. This level of oxygen flow was driving off CO_2 in sufficient enough volumes to increase the culture pH, which led to additional CO_2 sparging. The increased CO_2 sparging drove off oxygen, causing increased flow through the oxygen MFC. The competing oxygen and CO_2 control loops resulted in a cell culture environment that was suboptimal for cell proliferation, leading to a lower-than-anticipated maximum viable cell density and viability upon harvest. A well-trained operator would have noticed the CO_2 flow anomalies in real-time and could have corrected the problem on Day 3 by capping the oxygen flow through the drilled hole sparger and instead using a microsparger. This would have decreased the overall flow of gas into the reactor significantly, preventing the loss of control. If corrective action was implemented in a timely fashion, this run could have performed comparably to the development runs.

- A well-characterized manufacturing process with well-defined control limits for monitored inputs and outcomes exhibited gas flows, cell growth, and culture viability outside of control ranges. Trained operations staff noted the process anomalies and initiated an investigation, which resulted in the detection of a mycoplasma contamination prior to culture harvest which, in turn, prevented the exposure of the downstream processing equipment to the contaminated cell culture.

It is clear from these examples (and others encountered by the BPTG team) that the use of real-time data improves the possibility that a process upset can be caught early and the appropriate action taken to resolve or terminate a run. However, this is only true when manufacturing personnel are trained to understand process trends, interpret process data, and identify process anomalies. Staff should be able to track nutrient, metabolite, gas flows, pH and DO effectively enough to define the stage of the culture (e.g., log growth, stationary /production phase, apoptosis) and identify potential issues as they arise. Given that the cost for a single mammalian cell culture batch can be millions of dollars, the effective use of real-time process data is ultimately a cost-effective measure.