Scale-up of Fed-batch Mammalian Cell Culture Processes

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Scale-up of a cell culture process is seldom straightforward, more so when scaling up for the first time in a new facility. The recipe for a successful scale-up includes a thorough understanding of the cell culture process, manufacturability of the process at scale, well characterized bioreactors and efficient utilization of the available scale-up principles. Being a contract manufacturing organization, we have worked, and continue to work on different projects from different customers each with its own set of challenges. To tackle these distinct challenges it is important to have a holistic approach to scale-up which encompasses the aforementioned factors. In this review, we outline how this approach resulted in successful scale-up of multiple projects at Kemwell.

Any scale-up from a bench-scale bioreactor of a few liters to a final scale of thousands of liters in a cGMP facility is not a trivial task. The operational complexity further makes it challenging. But before diving into the scale-up, it is important to have a thorough understanding of the process at small-scale. Our scale-up team gets involved during the initial technology transfer from the client to the process development team. Being part of the tech-transfer team enables them to get a first hand look at the process, and evaluate the sensitivity of the process to hydrodynamic parameters such as aeration and agitation, environmental factors such as pH and dissolved carbon dioxide, and metabolic parameters such as lactic acid, all of which play a vital role when scaling up processes. Therefore, by developing an understanding of these parameters at an early stage coupled with testing for process robustness help significantly during scale-up. One important parameter that will be helpful during scale-up of different processes is the oxygen uptake rate commonly referred to as OUR. This information is useful during scale-up because the oxygen transfer capabilities of the bioreactor system should exceed or at the very least balance the OUR of the culture.

To ensure that the process developed at small-scale can be translated to the larger scale, the scale-up team together with the operations team assesses the manufacturability of the process. A facility-fit exercise aims to assess the fitment of the process with respect to existing equipment in the facility. The outcome of this exercise should likely result in a decision to purchase additional equipment to fit the process being developed at small-scale, or not. As a CDMO, we sometimes have to scale-up a process that is developed at the client's site and could involve an operation which could be complicated at scale, for example, the number of nutrient feed additions. If the number of feeds exceed the number of ports available, then additional connections may need to be made in a manner that the sterility is not compromised. Such modifications should preferably be verified in a pilot-scale system to ensure success at large scale.

With the variety of processes we encounter, it is imperative that we fully understand the capability of our bioreactors from bench-scale to large scale. So bioreactor characterization, which includes estimation of mass transfer coefficients for oxygen absorption and CO_2 desorption (kLa_{O2} and kLa_{CO2}) along with the estimation of bulk mixing time, is an integral part of scale-up. At Kemwell, a comprehensive characterization was done for bench-scale (5L glass), pilot (80L SS) and large scale (400L and 2000L SS) bioreactors. Model equations for kLa are derived for each of the bioreactors and this information becomes the basis for scaling up new processes.

Much information on mammalian cell culture scale-up is available in literature describing both the scale-up concepts as well as considerations. Volume independent parameters are straightforward to scale-up. In volume dependent parameters, scaling up aeration and agitation is much more challenging

due to the non-linear nature of these parameters. For example, scale-up of agitation speed based on tip speed alone can result in impractical agitation speeds at large scale. Similarly a linear scale-up of gas sparge flow rates may sometimes result in very high flow rates at large scale. At Kemwell, our strategy is based on using a “hybrid” approach for determining the agitation speed at large scale. This approach involves utilizing different parameters such as kLa, OUR, power per unit volume (P/V) in conjunction with the model equations for kLa. Based on the OUR information, the theoretical kLa required to satisfy the oxygen requirements at pilot-scale is calculated for example. Using the model equation for a 2000L bioreactor, the combination of P/V and sparge flow rates that could deliver the required kLa is iteratively calculated. Once the agitation speed and gas flow rates are determined, a few empirical parameters such as Kolmogorov eddies and gas entrance velocity are calculated using the agitation and sparge flow rates determined above to ensure cell shear is minimized.

Developing a thorough understanding of the process at small-scale while ensuring the developed process is scalable is very important for a successful transfer of biological processes to large scale. Coupled with leveraging the bioreactor characterization data available at all scales, the hybrid approach has been successful for multiple projects. Kemwell has successfully manufactured material for pre-clinical, clinical Phase I and Phase III studies at 400L and 2000L scales for India as well as US markets.