A Novel, Risk-Based Approach for Predicting the Optimum Set of Process and Cell Culture Parameters for Scaling Upstream Bioprocessing

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ABSTRACT

The ability to scale a cell culture effectively and efficiently, from lab to manufacturing, is critical to maximizing productivity whilst minimizing the risk of run failures and delays that can cost millions of dollars per month. The task of scaling well, however, is still considered to be a challenge by many upstream scientists, and this can be an exercise in trial and error. Traditionally, scaling has most often been performed using arithmetic in a spreadsheet and/or simple "back of an envelope" calculations. For some, it may even come in the form of support from a team of data scientists using advanced analytical software. This dependency on what some consider to be complex mathematics or statistics has resulted in the common consideration of using just one scaling parameter at a time, one scale at a time. However, it is difficult to determine easily or optimally, from the start, whether a process successfully transfers across scales based on only one process parameter, at one scale.

In this article, we describe the benefits of using a risk-based approach to scaling, and the development of a software scaling tool known as BioPAT® Process Insights for predictive scale conversion across different bioreactor scales. BioPAT Process Insights can be used to consider multiple parameters and across multiple scales simultaneously, from the start of a scaling workflow. We briefly describe how it was used in a proof-of-concept scale-up study to allow a faster, more cost-effective process transfer from 250 mL to 2000 L. In summary, using BioPAT Process Insights, in conjunction with a bioreactor range that has comparable geometry and physical similarities across scales, has the potential to help biopharma manufacturing facilities reach 2000 L production-scale volumes with fewer process transfer steps, saving both time and money during scale-up of biologics and vaccines.

INTRODUCTION

With speed-to-clinic and speed-to-market becoming increasingly critical to the delivery of biologics and vaccines, developing rapid methods to scale a cell culture process between bioreactors has come into sharp focus. The ability to select an optimum set of process parameters that scales up from laboratory through larger pilot-scale and finally, production-scale bioreactors is imperative to reduce the risk of process performance differences upon scale-up, and similarly vice versa for scale-down.[1] Scaling effectively and efficiently can also help maximize production yield and product quality. Sub-optimal selection of process parameters when transferring to the next scale can have a negative impact on key process indicators (KPIs) such as viable cell concentration (VCC), cell viability, cell diameter, and product titer. Similarly, critical quality attributes (CQAs) of a biologic, such as its glycan profile[2], can also be detrimentally affected.

To address significant process discrepancies, scientists typically need to undertake up to three engineering runs and one lock-down run to optimize process performance, which can take several months. The cost of re-runs during process transfer can be high, especially at manufacturing scales where the costs of media alone can run into thousands of US dollars. In addition, if process transfer causes production delays, one study estimates that for a biologic with $1 billion sales annually, it can cost up to $80 million for each month[3] of delay and emphasizes why scaling well is so important.

To help minimize costs and bioprocessing scientists' time, scale-up studies to select the best performing clone and then optimize process development are typically run in small, lower cost-per-run model systems. These include shake flasks and automated miniature bioreactors.
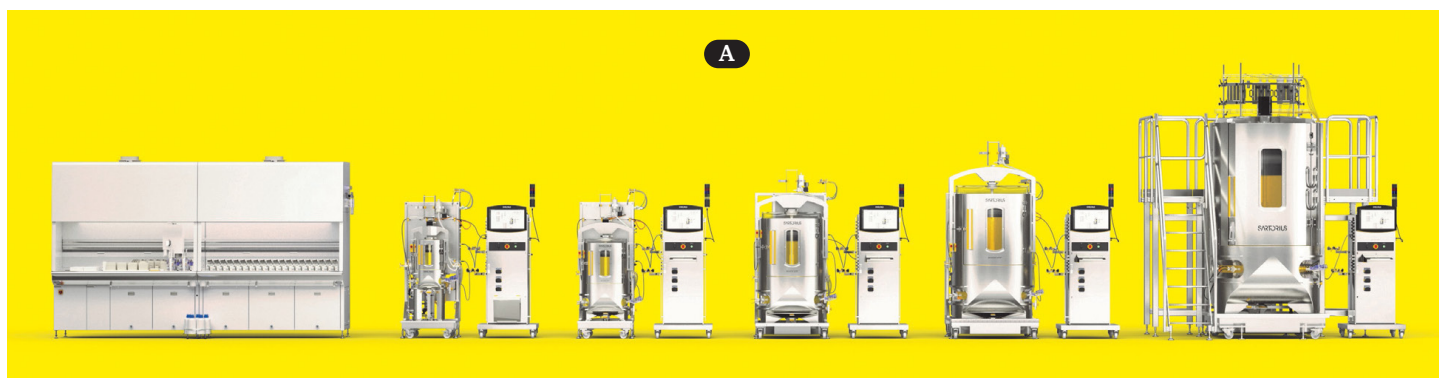
High-throughput, miniature bioreactors are commonly used in manufacturing settings for process development and clone selection studies. Bristol-Myers Squibb, for example, generated comparable profiles of cell growth and protein production to those seen at 5 and 1000 L bioreactor scales.[4] The paper indicated that benchtop bioreactor "mimics" translate well into manufacturing scale bioreactors. Being able to select clones and optimize a process using miniature bioreactors has helped significantly reduce process transfer timelines at the beginning of scale-up. In another example, scientists at Cobra Biologics reported that one of their process development projects was reduced from many months to just six weeks using automated miniature bioreactors in place of shake flasks and other benchtop bioreactors.[5]

Having the ability to scale down a suboptimally performing production-scale process by using a small bioreactor model is important for verification purposes. Process scientists at Merck demonstrated that high-throughput miniature bioreactors can be used as qualified, scale-down models for two monoclonal antibody (mAb) commercial processes at scales >10,000 L.[6]

FIGURE 1.
(A) A range of geometrically similar Sartorius single-use bioreactors (Ambr to Biostat STR).
(B) Schematic showing how a range of stirred bioreactors maintain a linear relationship with respect to height and impeller size.

LINEAR SCALING

To simplify scaling, the strategy of using a range of stirred tank bioreactors having comparable geometric and physical similarities across scales is shown in **Figures 1 A** and **B**. For example, the Ambr® 250 miniature bioreactor (Sartorius) has comparable impeller configurations and geometric similarities (vessel shape, height-to-diameter ratio, and impeller dimensions) to the Biostat STR® pilot and commercial-scale single-use bioreactors from Sartorius.[7,8] The Biostat STR range has also been designed with scaling in mind. For example, the hole count in the micro-sparger increases linearly with vessel height, from 50 L up to 2000 L.[9] Having detailed knowledge of all the vessels that will be used throughout the scaling workflow allows upfront consideration of their capabilities, ensuring that calculations of process settings (*e.g.,* stir speeds) are made according to each bioreactor's physical characteristics.

However, this strategy of using physically comparable stirred bioreactors for scaling, even when the range is designed to facilitate process transfer, is only half the story. Many parameters that are critical for process execution, such as shear and oxygen availability, have complex, non-linear dependenci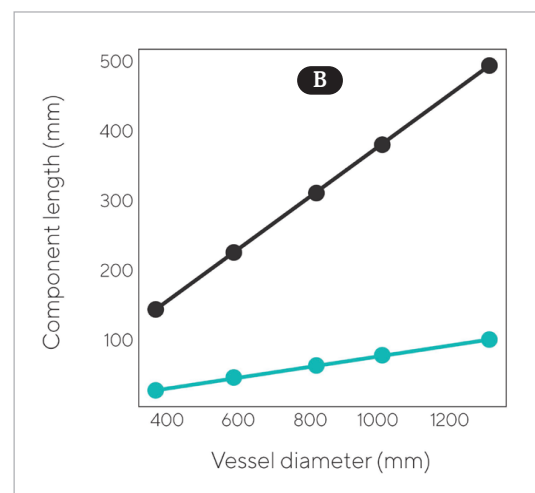es on bioreactor scale. Therefore, scaling can be further improved by ensuring that scale-dependent bioreactor settings like stir speed, fill volume, and gassing rates are chosen to be optimal across all scales. This involves using a quality by design (QbD) approach where scale-independent parameters such as specific power inputs (*e.g.,* power per volume [P/V]) and mass transfer coefficients (k_La) (*e.g.,* oxygen transfer) are conserved in larger bioreactors[10] and scale-down models.[11] Typically, bioprocess scientists choose one of these parameters and then select a bioreactor setting (*e.g.,* stir speed) so that the bioprocess parameter is the same between small- and large-scale bioreactors.

This can cause issues when developing a process across multiple scales from process development (*e.g.,* 250 mL) to production (*e.g.,* 2000 L). In some cases, a working P/V parameter in 2000 L scale can lead to sub-optimal conditions (*e.g.,* low stirring speeds) in miniature bioreactors. To improve this scaling approach, scientists should evaluate multiple bioprocess parameters and bioreactor settings simultaneously, from the beginning. They can try to adjust parameters like stir speed and gassing rates so that the P/V and k_La map more accurately across scales. This increases the chance of spotting high-risk transitions early on, enabling the scientists to respond appropriately in their scaling approach so as to minimize the risk of costly production-scale product failures down the road.

In this article, the benefits of a risk-based approach to scaling and the development of a software-based scaling tool known as BioPAT® Process Insights (Sartorius) to implement this approach are detailed. This software tool allows intelligent trade-offs whilst simultaneously taking other potential knock-on effects into account. We also briefly describe how it was used in a proof-of-concept scale-up study to allow a faster, more cost-effective process transfer from 250 mL to 2000 L.

METHODS

What is a Risk-Based Approach to Scaling?

Bioprocess scientists often describe how risk relates to bioprocess parameters. For example, a long mixing time can create a high risk due to the development and maintenance of heterogeneities within the bioreactor. Additionally, a high tip speed may come with more risk due to the larger shear forces it creates within the bioreactor. The degree of risk is cell-line and process dependent and is informed by experimental evidence from design of experiments (DoE) and scientific insight.[12,13]

Risk in bioprocessing can be described in absolute terms: for example, a high tip speed is high risk. Alternatively, risk can be expressed in relative terms such that if the power input P/V changes significantly or the k_La decreases during process transfer, from small to large scale, this can translate as high risk (**Figure 2A**).

By empirically characterizing a set of bioreactors in terms of the mapping between bioreactor and bioprocess parameters (**Figure 2B**) it is possible to generate a dataset and create statistical models
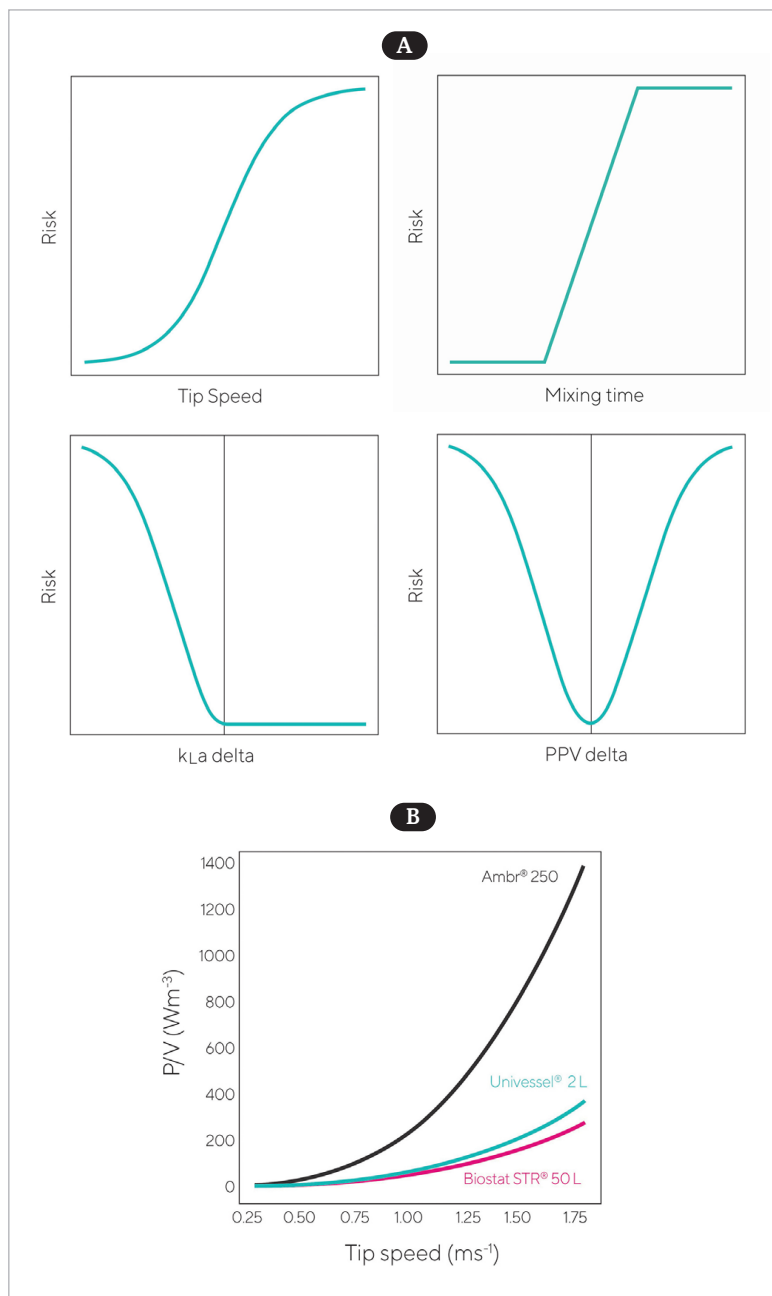

FIGURE 2. Graphs showing: **(A)** different risk profiles with process parameters; and **(B)** how a range of bioreactors map to different process parameters.

which can then be used to determine, for example, the P/V at a given stir speed and fill volume for a specific bioreactor.

Risk-Based Approach to Single Bioreactor Scaling

To implement risk-based scaling, bioprocess parameters of a bioreactor should first be characterized to give an indication of how they behave across different process conditions like fill volume, stir speed, and gassing rate combinations. With candidate bioreactor conditions in mind for a particular scale, a risk analysis of each parameter is performed and then combined to

provide an aggregate risk score of all parameters. Implementing this approach (**Figure 3A**) allows a risk profile map (**Figure 3B**) to be generated, taking into consideration all parameters and any potential knock-on effects.

This risk-based approach to scaling produces an optimal protocol for running the bioreactor (taking everything known about the process and bioreactor into account, including any constraints) and helps determine the sensitivity of the process, and therefore how much the bioreactor parameters can be varied without incurring significantly more risk. The risk map generated provides an indication of the operational space, enabling bioprocess scientists to determine a set of parameter values at which they can run their bioreactor with less risk than their acceptable threshold.

Risk-Based Approach to Multiple Bioreactor Scaling

Scaling across multiple bioreactor sizes requires implementation of an algorithm that copes with multi-dimensional optimization of parameters, at various scales and with non-linear response surfaces, due to the complex interaction between the scale-independent (bioreactor) and scale-dependent (bioprocess) parameters. Applying this algorithm works best when evaluating more process considerations in different bioreactor scales. To do this requires the characterization data of a bioreactor portfolio (*i.e.,* covering multiple scales) in terms of mapping from bioreactor and bioprocess parameters, across thousands of process conditions with data generated using consistent methodology.

In practice, when determining how to run a mini bioreactor (250 mL), there is plenty of flexibility with stirring speed, gassing, and fill volume at this scale, but for confirmatory tests using a benchtop bioreactor (2 L scale), there may be less flexibility if, for example, there are other constraints on fill volume. Then when transferring the process to 2000 L via a 50 L scale, it may be the case that only stirring speed can be adjusted in the context of a platform process that mandates a particular gassing strategy. To future-proof the process at scale, more consideration is needed when choosing settings for the miniature bioreactor scale due to the lack of flexibility at larger scales. This results in the creation of a network of considerations (**Figure 4**).

Using the traditional approach to scaling, with its network of considerations, can be time consuming and complicated because it requires multiple upfront calculations. This can involve the use of in-house
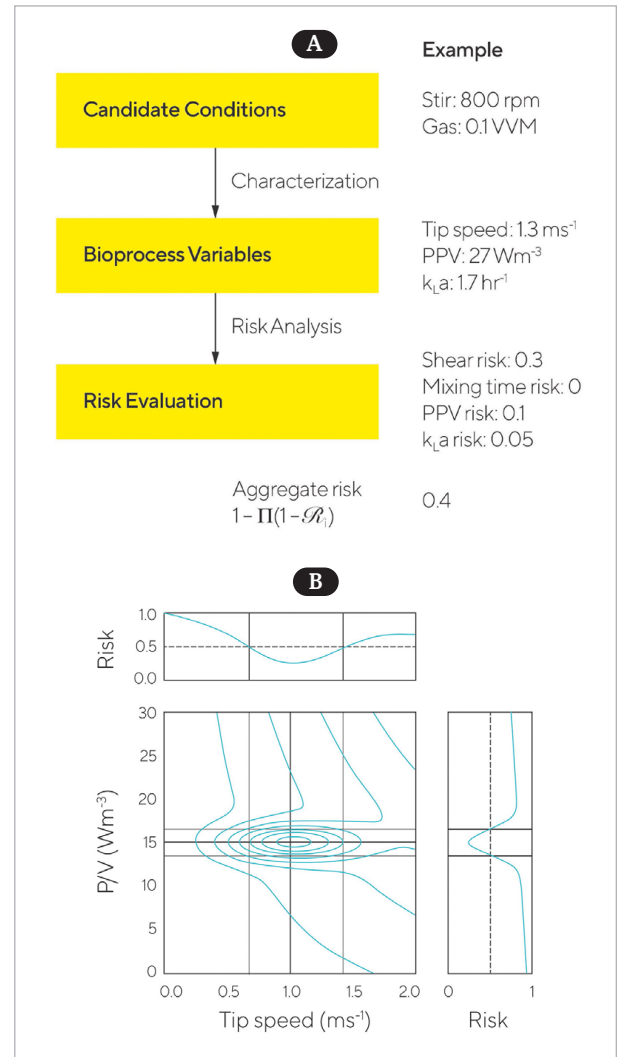
FIGURE 3. Schematics showing: **(A)** an example calculation of how cell culture conditions, process parameters, and evaluating risk can generate risk profile data; and **(B)** risk profile map generated statistically from dataset (using calculations from Figure 3A).

FIGURE 4. Example network of process considerations when scaling from mini to commercial single-use bioreactors.
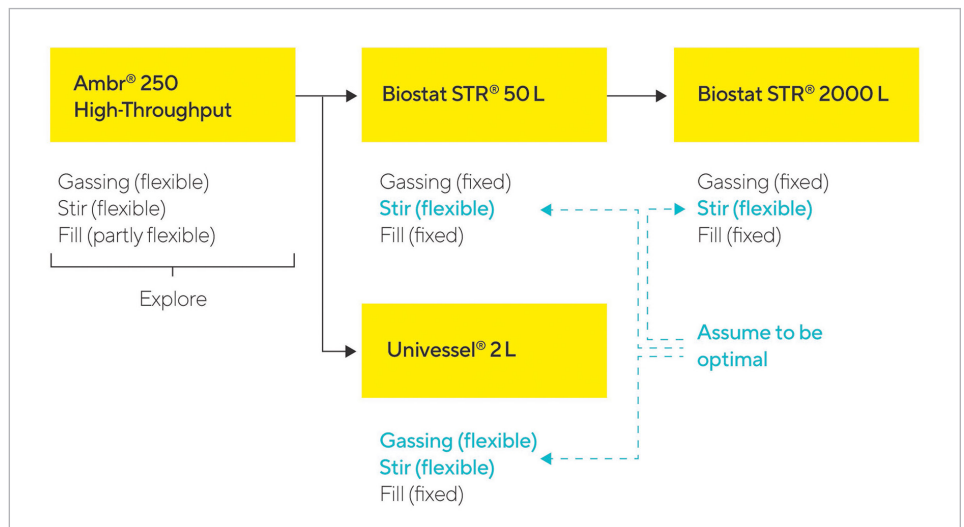
spreadsheets or relying on a team of data scientists to perform the analysis using advanced analytical software. Whichever the method, vast amounts of gathered bioreactor data are required to provide a more accurate calculation beyond theoretical relationships. "The more data, the better" cannot be underemphasized. While the amount is critical, so is the quality and completeness of the data, and this is determined by how this data was obtained. For scaling, characterized bioreactor data should be measured consistently across scales using the same methodology. Typically, unless bioreactor scientists have the time and resources to perform all their engineering runs across scales using the same methodology, many datasets will instead include a mixture of data from different methodologies, vendors, public domain, and historical engineering runs. There might also be gaps in the datasets.

Predictive Process Scaling Streamlined

Sartorius has developed the software scaling tool, BioPAT Process Insights, to automate risk-based scaling for its single-use bioreactors from 250 mL up to 2000 L. This tool provides bioprocess scientists with a guided user interface embedded with bespoke underlying technology to scale more easily, effectively, and efficiently across multiple parameters and scales simultaneously. Within

the tool is a characterized database that Sartorius has generated from Ambr and Biostat STR single-use bioreactors across thousands of process conditions using consistent DECHEMA protocols.[12] With BioPAT Process Insights, scientists can generate risk maps indicating optimal conditions, operational space, and sensitivity at the 250 mL scale with subsequent scale projections in the process workflow. "Sweet spots" can be rapidly identified for all bioreactors across the workflow to operate at their maximum potential.

Proof-of-Concept

Using a risk-based approach with the BioPAT Process Insights software scaling tool, proof-of-concept cell culture process parameters for a CHO cell line expressing a commercial mAb were generated. Process parameters were then used for experimental scale-up studies[14] in a miniature bioreactor (250 mL), a benchtop bioreactor (2 L), and larger scale (50 L, 200 L, and 2000 L) bioreactors. Stirring speeds were set according to the lowest risk profile generated to correlate with a Reynolds number (>3,000), tip speed (0.6–1.25 m/s), and a specific power input of 30–200 W/m³ across scales. Comparable performance in cell growth, metabolic profiles (**Figures 5A** and **B**), and product quantity (not shown) was achieved at all scales.[14]
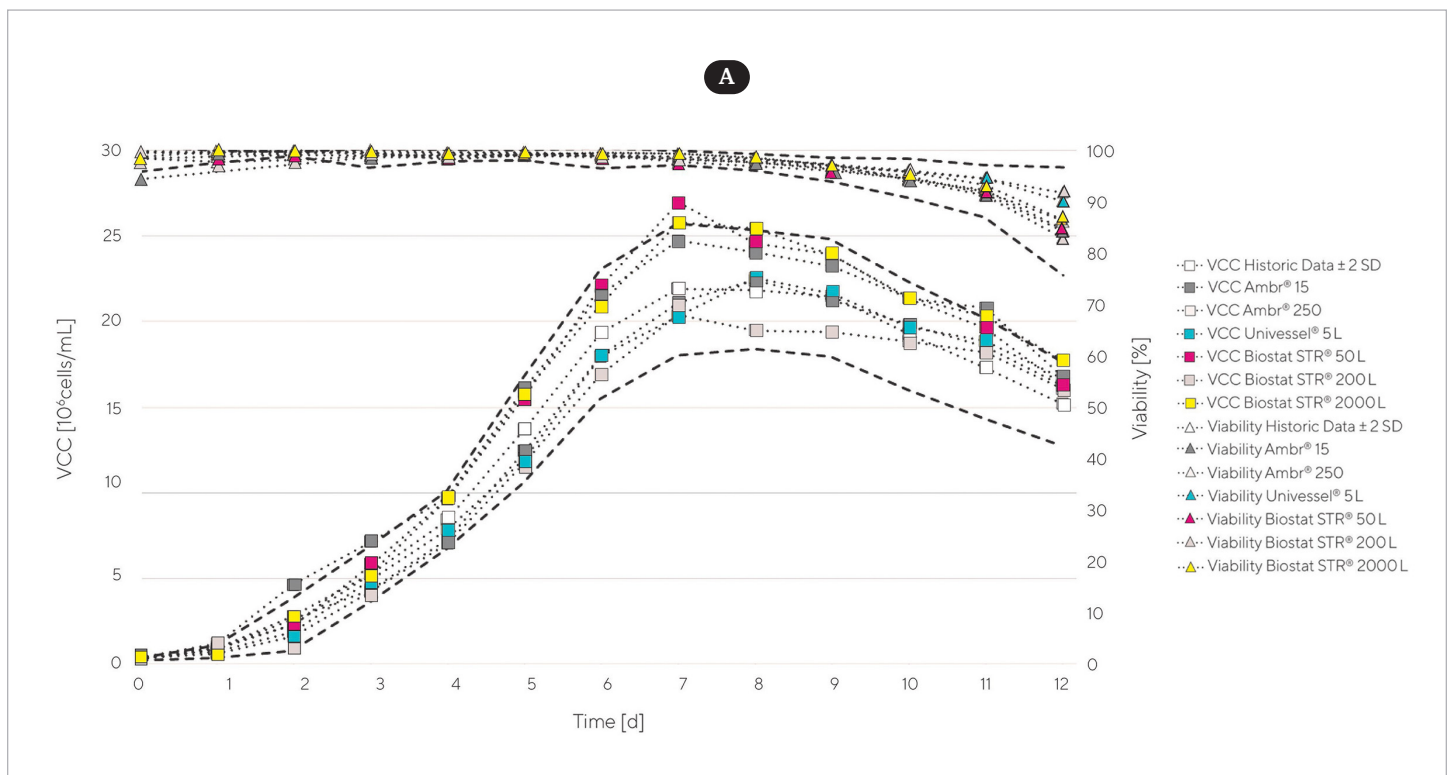
FIGURE 5A. Graph showing VCC and viability in CHO cells cultured in bioreactors from 250 mL to 2000 L. (Dashed black lines represent historical golden batch data for each parameter measured ±2 SDs.)

FIGURE 5B. Graph showing glucose, lactate, and osmolality profile in CHO cells cultured in bioreactors from 250 mL to 2000 L. (Dashed black lines represent historical golden batch data for each parameter measured ±2 SDs.)
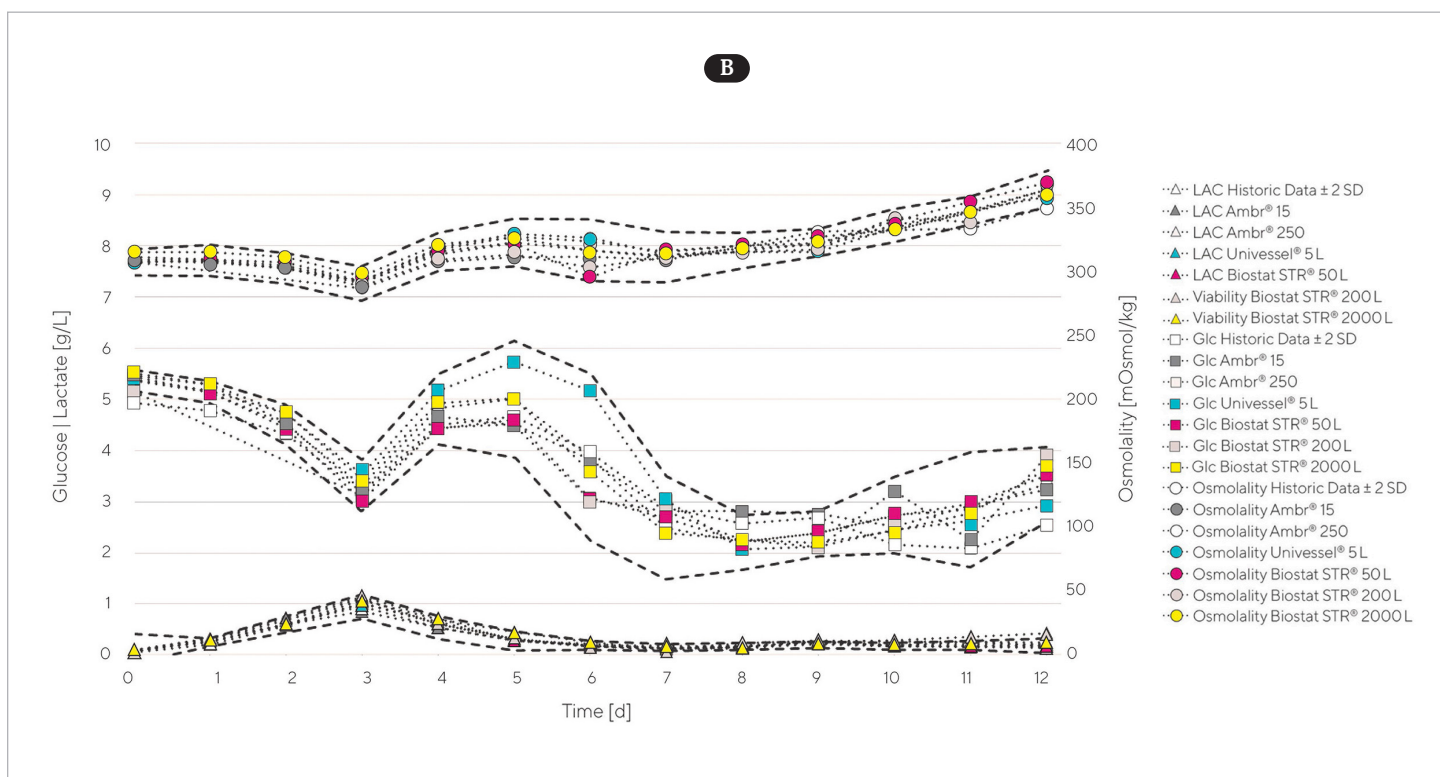
CONCLUSION

Conducting traditional calculations across multiple scales takes considerable time and effort, and although they can often provide good results, optimal results are not always realized. Depending on the bioreactor data generated using inconsistent protocols or theoretical relationships versus empirically-derived data can lead to wasteful inconsistencies. This article has described how to improve process scaling by evaluating multiple bioprocess parameters and bioreactor settings simultaneously, using a risk-based approach to produce a network of process considerations.

Using a newly developed software application called BioPAT Process Insights, a risk map of optimum conditions, operating space, and sensitivity at the 250 mL bioreactor scale was generated for a well-characterized stirred tank CHO cell culture, with projections of how this would appear at each bioreactor scale from 5 L to 2000 L. The predicted process and cell culture conditions were tested experimentally and found to have comparable performance at all scales.

In summary, using the BioPAT Process Insights software in conjunction with a bioreactor range that has comparable geometry and physical similarities across all scales can save substantial time and financial resources in the development of scalable cell culture processes. Streamlined scaling can accelerate time to clinic, as well as market access to products such as therapeutic biologics and life-saving vaccines.

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