Simplifying Progress

A Rapid, Low-risk Approach for Process Transfer of Biologics from Development to Manufacturing Scale

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Abstract

Manufacturing of biologics commonly focuses on an upstream process with Chinese Hamster Ovary (CHO) cells in a production bioreactor. Before this can begin, scientists have to find the best culture parameters using shake flasks or mini bioreactors. They then transfer these parameters from the laboratory through different scales of bioreactor. Typically, process transfer during scale-up involves transferring through several different pilot and manufacturing scale bioreactors and doing multiple iterations to optimize process parameters at each scale, all of which is expensive and can take many months. BioPAT® Process Insights is a software tool that can be used to minimize the risks associated with conventional bioreactor scaling and design space exploration. The software combines scaling functionality with characterized bioreactor data to support consistent scale up between Sartorius bioreactors. This study shows it is possible to scale up a cell culture process from 15 mL through pilot scale and up to 2,000 L without affecting the process or product quality.

Ambr® 15 Cell Culture (15 mL) | Ambr® 250 High Throughput (250 mL) | Univessel® Glass (5 L) | Biostat STR® Generation 3 (50 L) | Biostat STR® Generation 3 (200 L) | Biostat STR® Generation 3 (2,000 L)

Materials and Methods

Cell Line: Cellca CHO DG44 expressing a human IgG1 mAb.
Bioreactors: Ambr® 15 Cell Culture, Ambr® 250 High Throughput, Univessel® 5 L, Biostat STR® 50 L, 200 L, and 2000 L.
Sensor: BioPAT® Viamass sensor (used for the Univessel® 5 L, Biostat STR® 50 L, 200 L, and 2,000 L).
Software: Bioreactor scale-up with BioPAT® Process Insights, Multivariate data analyis with SIMCA®
Process Setpoints: Temperature 36.8°C, pH 7.1, DO 60%.
Process set up: 12-day fed-batch culture with sampling for metabolites, VCC, viability, titer, and N-glycans for all bioreactors.

Bioreactor Agitation Rates

Bioreactor agitation rates were set according to the BioPAT® Process Insights software 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 (Table 1).

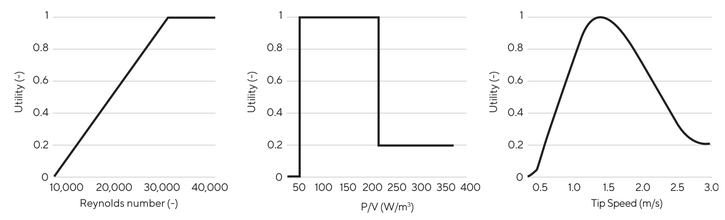
Table 1

Bioreactor Agitation Parameters

Bioreactor	Reynolds Number	Tip Speed (m/s)	Specific Power Input W/m³	Agitation Rate (rpm)
Ambr® 15 (15 mL)	3638	0.66	190	1050
Ambr® 250 (250 mL)	13910	1.252	183	855
Univessel® SU (5 L)	38563	1.252	54.1	327
Biostat STR® (50 L)	79728	1.252	30.4	162
Biostat STR® (200 L)	147427	1.254	30.5	121
Biostat STR® (2000 L)	407807	1.258	29.6	70

Output from the software shows Reynolds number (Re), tip speed and P/V changed according to agitation rate, with functions developed with a score from 0 to 1 over the range of the parameters, where 1 represents the optimum for each parameter (Figure 1)

Figure 1



Note. BioPAT® Process Insights software data showing the optimum Re, PPV and tip speed based on the agitation rate.

Process Transfer Performance

The DO and pH profiles across all scales are comparable (Figures 2A and 2B).
Temporary decreases in DO for the Ambr® is due to opening of the vessel during automated sampling and feeding.
Temporary increases in pH across all scales is due to the addition of a basic feed.

Figure 2A

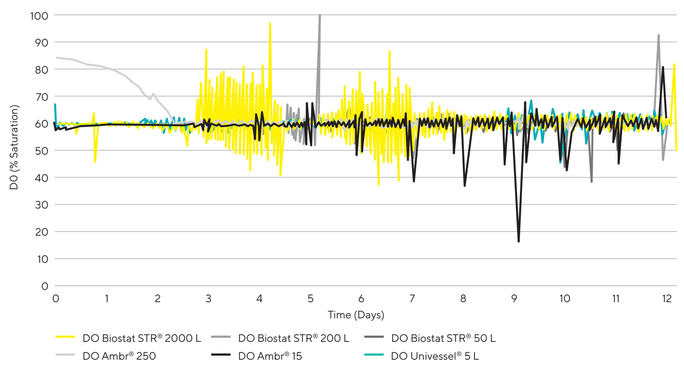
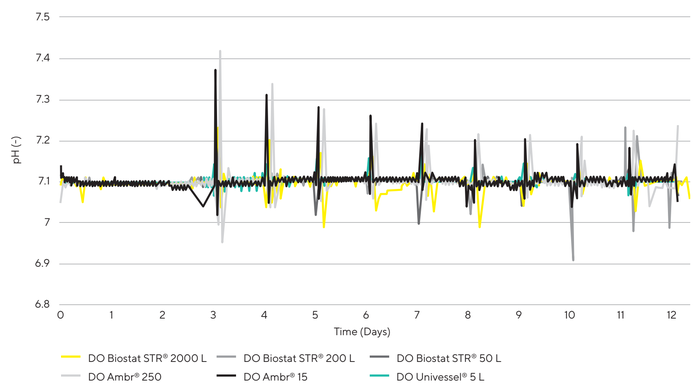


Figure 2B



Note. DO profile (2A) and pH (2B) across all scales over 12 days.

Bioprocess Performance at Different Scales

The CHO cell culture showed comparable performance with regards to cell growth, metabolic profiles and product quantity/quality at all scales.
Peak VCD of 20–26 x 10E6 cells/mL on day 7 to 8 with harvest viabilities between 80-90% (Figure 3).
Glucose, lactate, and osmolality profiles consistent throughout the run (Figure 4).
All results comparable to historical golden batch reference data (mean of 30 different Ambr® 250, Univessel® and Biostat STR® bioreactor sizes with CHO cells expressing a commercial IgG).

Figure 3A

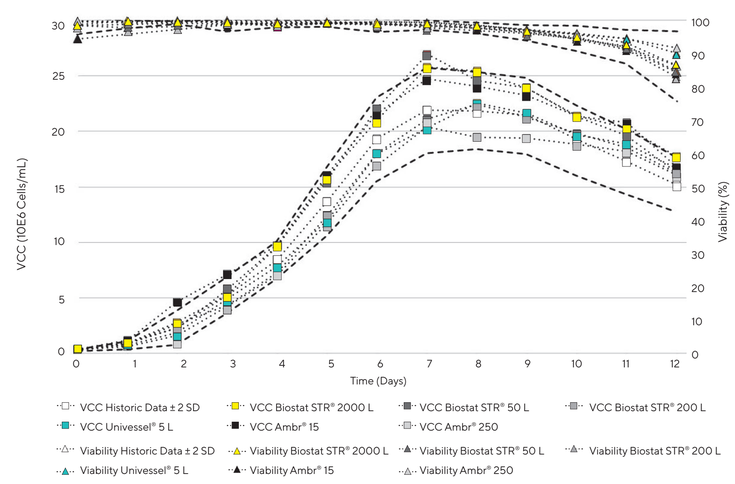
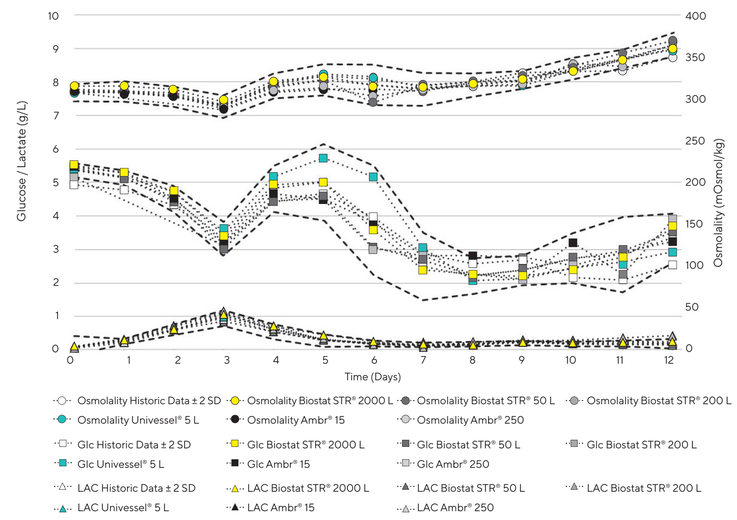


Figure 3B

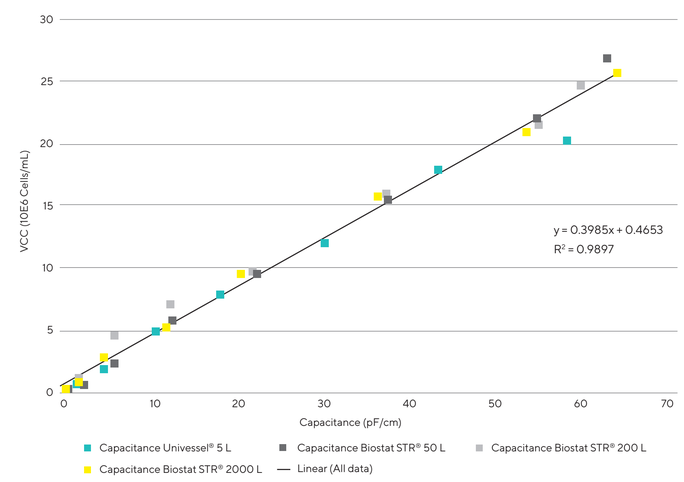


Note. VCC and viability (3A) and glucose, lactate and osmolality profiles (3B) across all scales over 12 days. Dashed black lines in Figure 3A and 3B represent historical data for each parameter measured +2 and –2 standard deviations.

Scalability of On-Line Analytics

Linear regression capacitance model constructed using online BioPAT® Viamass data measured at a single-frequency indicates transferability between scales during the exponential growth phase with an R^2 value of 0.99 (Figure 4).

Figure 4



Note. Linear regression model of capacitance and VCC over 12 days for 5 L to 2,000 L bioreactor scales.

mAb Titer and Critical Quality Attributes

The daily and total product concentrations showed comparable trends across all scales with harvest concentrations of 2.5-3.5 g/L being achieved on day 12 (Figure 5).
Critical Quality Attributes (CQAs) of the mAb were not affected during process transfer and showed similar trends in glycosylation patterns of G2/G0 and G1/G0 ratios and comparable binding potencies with between 85-90% fucosylated forms produced from cells cultured from the complete bioreactor range (Figure 5).

Figure 5A

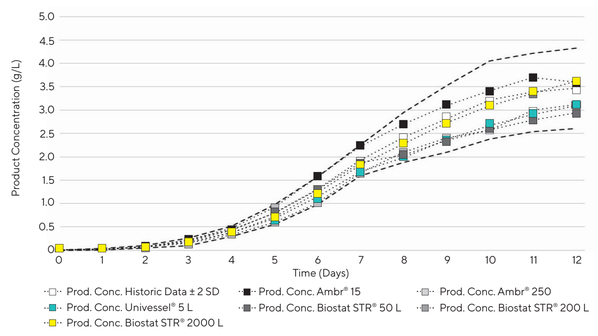
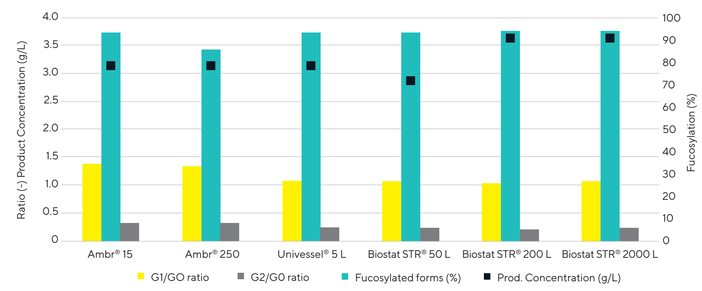


Figure 5B



Note. Daily product concentration (5A) and cumulative product concentration, glycosylation and fucosylation profiles (5B) across all scales over 12 days. Dashed black lines in Figure 5A represent historical data +2 and –2 standard deviations.

Principle Components Analysis

Principle Component Analysis (PCA) was run using SIMCA® Multivariate Data Analysis (MVDA software with VCC, viability, glucose, lactate, osmolality, cell diameter, product quality and final titer data from the Ambr® and Biostat STR® bioreactor runs).
This analysis demonstrates that small scale batch data clusters closely together near to the center of the plot with larger scale bioreactors clustering together on one side of the plot due to their generally higher VCCs and titers.
All batch data are within the 95 % confidence region, indicating scalability of the process from 15 mL to 2000 L. (Figure 6).

Figure 6

Note. Batch level model generated on the basis of a PCA of bioprocess data across all scales.

Conclusion

Utilizing the BioPAT® Process Insights software, it possible to scale up a cell culture process from the process development scale (Ambr® multi-parallel bioreactors and Univessel® benchtop bioreactors) to pilot and commercial manufacturing scale (Biostat STR®) without affecting the process or product quality.

Using this scale-up approach offers bioprocess scientists the potential for developing faster, more cost-effective cell culture processes for their biologics manufacturing