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SPECIAL REPORT

Cell Culture Scale-Up in Stirred-Tank Single-Use Bioreactors

BY MICHELET DORCEUS

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Bioprocess development usually is carried out in systems with small working volumes. This helps save time and resources because, at small scale, several experiments can be conducted in parallel. Costs for media are kept low, and relatively little laboratory space is required to operate small-scale bioreactors. But over the course of development, biopharmaceutical companies need more material for characterization, trial runs, and finally for commercialization. They transition to bench scale and then up to pilot or production scale with the intent to maintain constant yield and constant product characteristics. Often that brings some challenges.

To deal with those, development groups must consider shear-stress levels, concentration gradients, and oxygen supply capabilities of production bioreactor systems during scale-up. One important factor is the culture mixing time. As cultures increase in scale, developers want to ensure consistency in mixing because that can influence cells' physiology. Therefore, both product quality and yield can be influenced by gradients in glucose concentration, oxygen, and pH. As companies scale up to pilot/production bioreactor(s), they need to maintain culture homogeneity to ensure an ideal growth environment at all scales.

Key **process engineering parameters** related to scale-up include power input/volume ratio (P/V), impeller tip speed, constant mixing time, constant volumetric mass transfer, and constant oxygen transfer rate (OTR). The latter is an important factor for aerobic cultures. The volumetric mass transfer coefficient ($k_L a$) describes the efficiency with which oxygen can be delivered to a bioreactor culture for a given set of parameter conditions. For proper cell culture scale-up, it is important to select equipment of different sizes with similar $k_L a$ capabilities so that small-scale success can be replicated at larger scales. Mixing time is another important factor. As you scale up, you want to prevent concentration gradients and achieve homogeneity throughout each vessel.

Key process engineering PARAMETERS related to scale-up include power/input volume ratio, impeller tip speed, constant mixing time, constant volumetric mass transfer, and constant oxygen transfer rate.

Impeller tip speed influences mixing time and oxygen transfer as well as how cells are kept in suspension. Usually tip speed (rather than agitation speed) is kept constant across scales. The tip speed is equal to $\pi \times d \times N$, with d being the impeller's outer diameter (m) and N being the agitation speed (rps). However, shear forces increase with increasing tip speed. Thus, it is important to find a suitable tip speed that allows sufficient mixing and oxygen transfer but does not cause too much shear stress to the cells. Keeping tip speed constant maintains a relatively constant shear force level, but it may reduce mixing-time performance in large-scale vessels.

The power input/volume ratio (P/V) can influence oxygen transfer and culture mixing, as well. P/V can be converted from the impeller power number using the following equation:

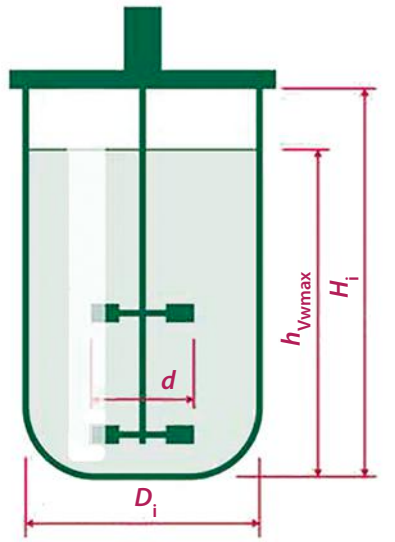
$$P/V = (N_p \times \rho \times N^3 \times d^5)/V$$

with N_p being the impeller power number, ρ the density of water (1,000 kg/m^3), N the agitation speed (rps), d the impeller outer diameter (m), and V the vessel's full working volume (m^3).

In scale-up practices, power numbers are often determined without gassing. However, gassing greatly reduces impeller torque, thus having a significant impact on the apparent impeller power numbers as well as the results of aerobic processes. Maintaining constant P/V between vessels is one of the most common and prevalent strategies for scale-up.

Most scale-up strategies include the goal of keeping one or more parameters constant across scales. Which parameter is most important depends on the process. It is important to scale up your process using vessels with similar geometries and similar conditions (e.g., in terms of stirrer type, installation conditions, and process parameters such as filling rate, aeration rate, and type of gassing device and pressure).

Figure 1: Stirred-tank bioreactor design



Stirred-Tank Bioreactors

The stirred-tank bioreactor design is relatively easy to describe with classical engineering approaches (Figure 1). Parameters to describe this vessel geometry include impeller diameter, vessel diameter, liquid height, and ratios thereof. It is the bioreactor design for which most research on

Figure 2: The BioBLU c single-use vessel portfolio

BioBLU Model	0.3c	1c	3c	10c	50c
Working Volume	100–250 mL	320 mL–1.25 L	1.25 L–3.75 L	3.5 L–10.0 L	18 L–40 L

Figure 3: $k_L a$ values for different BioBLU single-use vessels under different experimental conditions

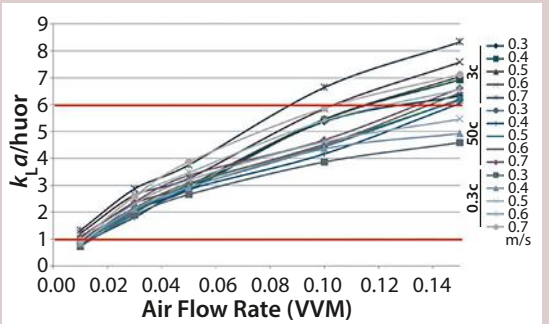
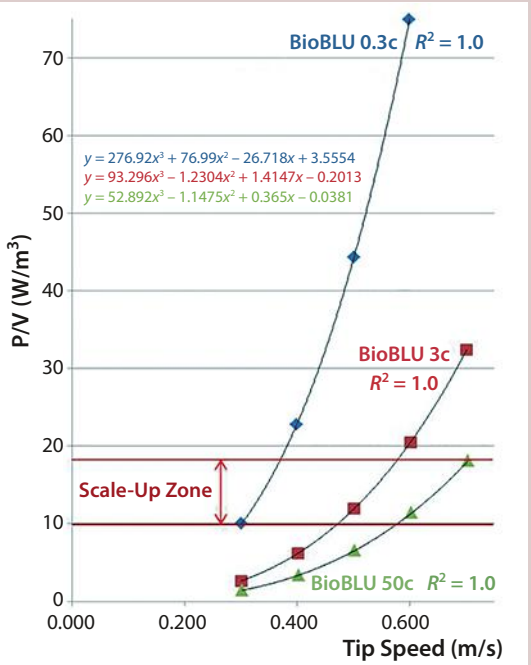


Figure 4: P/V scalable zone



scale-up phenomena has been conducted. This knowledge has been transferred into single-use technology, as well.

BioBLU single-use vessels have rigid walls to combine the best of both (single- and multiuse) worlds. They have an industrial rigid-wall design for supporting scale-up within the BioBLU portfolio and beyond. They provide fast and efficient mixing with magnetically coupled overhead drives. And BioBLU single-use vessels are made of USP class VI certified material free of animal components. The BioBLU c single-use vessel portfolio offers the widest range of rigid-walled, single-use, stirred-tank bioreactors available (Figure 2), with a working-volume range from 100 mL to 40 L.

Vessel geometry is similar across scales in terms of (among other things) the ratio of impeller diameter to vessel inner diameter and the ratio of maximum liquid height to vessel inner diameter. This facilitates use of BioBLU vessels at small scales during process development and then scale up processes to larger working volumes. Knowing the vessel characteristics and using the formula described above, we have calculated the tip speed at different agitation speeds for each BioBLU c single-use vessel. Thus, we have defined a scalable tip speed zone of 0.1–0.7 m/s, which is reachable by all vessels.

Case Study #1

A case study of monoclonal antibody (MAb) production using BioBLU single-use vessels was performed at our application laboratory in Enfield, CT, USA. We investigated the capabilities of Eppendorf BioBLU single-use vessels for cell culture scale-up from small to pilot scale. We took this process from a small-scale BioBLU 0.3c (250 mL maximum working volume) and scaled it up 10-fold to the BioBLU 3c bench-scale bioreactor (3.75 L maximum working volume) and then up to the pilot-scale BioBLU 50c (40 L maximum working volume). We used a DASbox Mini Bioreactor system to control the BioBLU 0.3c process; to control the BioBLU 3c and 50c processes, we used a BioFlo 320 bioprocess control station.

We investigated the $k_L a$ capabilities of each vessel using the static gassing-out method. For each vessel, we determined the $k_L a$ at tip speeds between 0.3 and 0.7 m/s and gas flow rates between 0.01 and 0.15 vessel volumes per minute (VVM). For the different experimental conditions, we achieved a wide range of $k_L a$ values that we plotted to determine the $k_L a$ -scalable zone. As illustrated by the two orange-colored lines in Figure 3, $k_L a$ values of 1–6 h^{-1} are reachable by all vessels.

We planned to scale up the MAb production process based on constant P/V. There are two methods to determine the power number: computational fluid dynamic (CFD) analysis and experimental measurements. In our first case study, we determined the vessels' power number based on CFD analysis. For the BioBLU single-use vessel, we assumed one impeller with up-flow mixing and no gassing. For each vessel type, we determined the power number at tip speeds

Figure 5: Chinese hamster ovary (CHO) cell growth

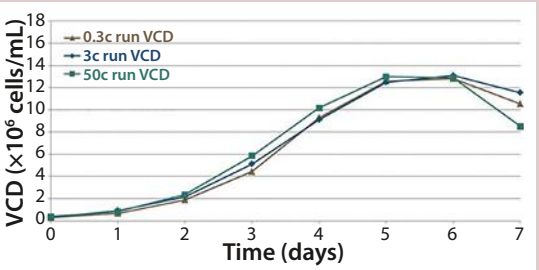
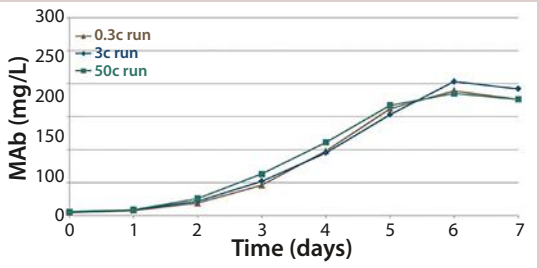


Figure 6: Monoclonal antibody (MAb) production



between 0.3 and 0.7 m/s. The mean power number calculated from that was 2.54 for the BioBLU 0.3c single-use vessel, 2.56 for the BioBLU 3c single-use vessel, and 2.52 for the BioBLU 50c single-use vessel. Then we calculated P/V using the equation described above and plotted it against the tip speeds, which helped us to establish a scale-up zone of 10–18 W/m^3 (Figure 4, between the two red lines).

Based on those data, we chose a P/V of 10.9 W/m^3 for our study. We used an air flow mode of three-gas autocontrol with a maximum gassing of 0.19 VVM, a pH setpoint of 7 with a 0.1 deadband, a dissolved oxygen (DO) setpoint of 50%, and an operating temperature of 37 °C. The working volume at small scale (BioBLU 0.3c) was at 0.25 L, 3.75 L at bench scale (BioBLU 3c), and 40 L at pilot scale (BioBLU 50c). At all scales, we used tip speeds that gave a P/V of 10.9 W/m^3 (BioBLU 0.3c: 0.3 m/s, BioBLU 3c: 0.47 m/s, BioBLU 50c: 0.58 m/s).

Chinese Hamster Ovary (CHO) Cell Culture Results: Figure 5 shows viable cell density (VCD) plotted against time for all three scales. Cell-growth patterns are very similar at all scales, indicating that we achieved suitable scale-up parameters with our single-use vessels to scale up the CHO process from a working volume of 0.25 L to 40 L.

To show the production yield (Figure 6), we plotted the MAb concentration in mg/L over time. Production profiles at the 0.25 L, 3 L, and 40 L working volumes were almost identical. That gives us the confidence to say that we chose suitable operating parameters to scale up our process from a working volume of 0.25 L to that of 40 L.

Case Study #2

A second case study was conducted at Cevec Pharmaceuticals GmbH. That company's bioprocess engineers wanted to take a viral vector production process from shake flasks to a BioBLU 3c (working volume of 2 L) and a BioBLU 10c (working volume of 10 L). They based their scale-up on power numbers determined experimentally by the Eppendorf applications laboratory using a torque meter. To do so, the BioBLU single-use vessels were modified to remove the magnets from their magnetic drive couplings. That way, the torque sensor could be directly connected to the impeller shaft. Our application scientists determined the power numbers at 0.3–1.0 m/s tip speeds and then calculated the average. The BioBLU 3c mean was 2.98, the BioBLU 10c mean was 3.33, and the 50c mean was 3.15.

Cevec Pharmaceuticals uses a unique human-cell–based expression system (CAP technology) in two product portfolios. The glycooptimized CAP-GO cell line for tailor-made production of N- or O-glycosylated proteins; the CAP-GT cell platform is for stable and transient industrial-scale production of recombinant adenoassociated viruses (rAAV) and for lentiviral and adenoviral gene-therapy vectors. In this study, the company scaled up a rAAV transient production process using CAP-GT cells expressing rAAV8-GFP through a two-plasmid system from PlasmidFactory. The researchers wanted to compare an initial shake-

Figure 7: Cell growth and viability

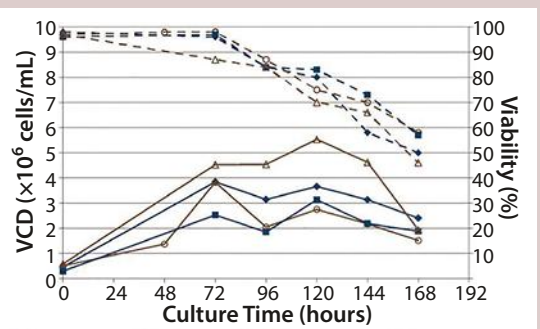


Figure 8: Transfection efficiency

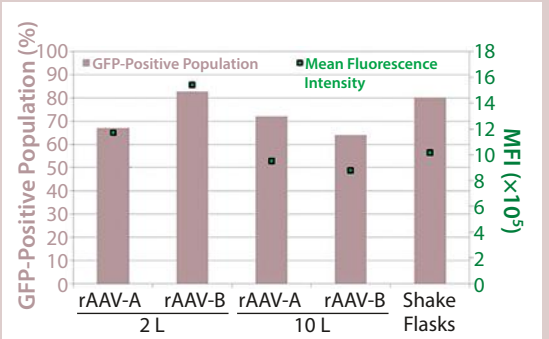
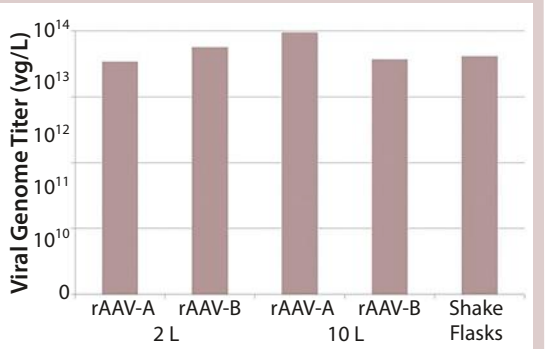


Figure 9: Productivity



flask process with scaled-up bioprocesses at working volumes of 2 L and 10 L.

For rAAV production, cells are expanded in suspension in a chemically defined medium free of animal components. Transient transfection takes place 72 hours into the process and is followed by a production phase and harvest. The BioFlo 320 bioprocess control station was used to control both the BioBLU 3c and BioBLU 10c vessels. The gassing strategy used an automatic gas mix that automatically controls air, oxygen, nitrogen, and CO_2 depending on the process parameter setpoints. For pH regulation, $NaHCO_3$ is added as a base and CO_2 gassing as an acid.

Scale-Up Strategy: Agitation was optimized during process development. Scale-up was based on similar power input at both scales. The agitation speed of the 2-L working volume BioBLU 3c vessel was set to 200 rpm (corresponding to a tip speed of 0.69 m/s); for the 10-L working volume BioBLU 10c, it was 175 rpm (corresponding to a tip speed of 0.84 m/s). Those settings provided for comparable power inputs of ~62 W/m^3 at both scales.

Cell Growth Results: The researchers conducted two bioprocess runs at the 2-L scale (2 L rAAV A and rAAV B) and two runs at the 10-L scale (10 L rAAV A and rAAV B). For all runs, they plotted VCD (solid lines) and viability (dotted lines) over time (Figure 7), with similar growth patterns achieved. VCD at the point of transfection was similar to that of the original shake flask process. Posttransfection behavior was similar as well. The DO control was very tight at both scales, with similar profiles for all four runs, and all four showed similar pH profiles.

The researchers determined transfection efficiency (Figure 8) based on green fluorescent protein (GFP) fluoresence measured with a NucleoCounter NC-3000 instrument from Chemometec. Similar transfection efficiencies were achieved at both scales. Transfection efficiency was comparable to that achieved with the original shake-flask process.

The bioprocess engineers at Cevec measured productivity (Figure 9) by quantification of genome titer by quantitative polymerase chain reaction (qPCR), and it was similar at both scales — again, comparable to results from the original shake-flask process. However, the team did notice some variability that may be attributable to the process and analytical method of the qPCR used to determine the viral genome titer.

Conclusion

In summary, we showed comparable BioBLU c vessel geometries and capabilities across scales. We successfully scaled up a MAb production process in CHO cells from the 0.25-L to the 3-L and then the 40-L scale. Using BioBLU 3c and 10c single-use vessels, bioprocess engineers at Cevec Pharmaceuticals scaled up a rAAV vector production process from shake flasks to 2-L and 10-L scales while achieving comparable transfection efficiency, cell growth, and productivity.

Michelet Dorceus *is a bioprocess field application manager at Eppendorf North America; bioprocess-info@eppendorf.de; www.eppendorf.com/bioprocess.*

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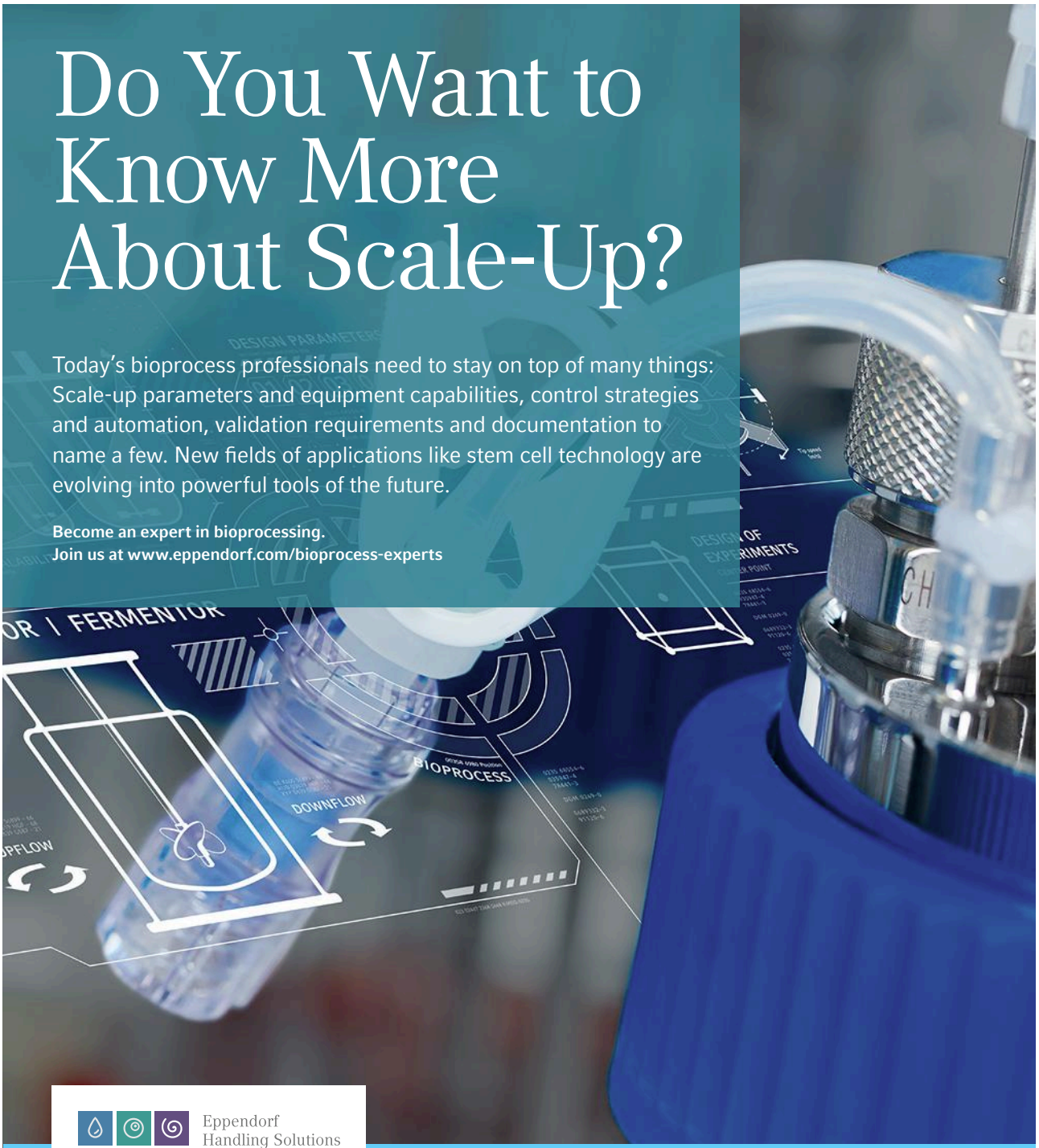

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