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Feliza Mirasol



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Bioprocessing Scale-Up Challenges and Eppendorf's Newest Solution: Thought Leader Q&A
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New Therapies Present Scaling Challenges

Feliza Mirasol

Manufacturing differences between traditional mAb therapies and newer biotherapeutics dictate whether processes should be scaled up, be scaled out, or use an alternative approach for commercial production.

The approval of emerging biologic-based therapeutics—such as cell and gene therapies—presents challenges and decisions for drug companies looking to increase the manufacturing volumes of these specialized therapeutics. However, differences in manufacturing cycles for emerging therapies, compared with that of the traditional monoclonal antibody (mAb) therapeutic, calls for different strategies to increase production.

In mAb production, increasing production volume to commercial scale is typically a scaling operation. Bioreactor size can be increased (scale up), or the number of bioreactors used can be increased (scale out). In addition, mammalian cell culture technology has advanced such that producing multi-kilogram quantities of therapeutic mAbs in one batch is now commonplace (1). With a cell therapy, however, establishing cost-effective ways to commercially manufacture cell therapies for mainstream use remains a challenge. For a gene therapy, some aspects of its manufacturing process can use a scale-up strategy similar to a mAb, but other aspects of its manufacture cannot be easily be scaled up.

The cell therapy challenge

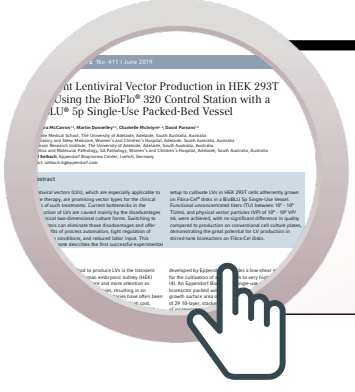
Cell therapies are generally divided into two categories: autologous and allogenic. Autologous cell therapies use a patient's own cells for the threapy, while allogenic cell therapies use cells

from a donor to develop a therapy to treat multiple patients. Specifically, autologous cell therapy manufacture is not easily scaled up; allogenic cell therapies have a better chance at following the traditional scale-up route.

With allogenic cell therapies, a single batch of donor cells is used to produce large volumes of the product for many patients, a model similar to traditional mAbs, in which a single cell-line source is cultivated to mass produce the product. The case for scaling up allogenic cell therapy production is thus supported by the economies of scale that apply to mAb production (i.e., scaling up production reduces the cost per dose of final therapeutic product) (2). In addition, both the engineering knowledge and operational expertise gleaned from scaling up mAb production already exists and can be used to support the increased manufacture of allogenic cell therapies, provided that a product quality profile for the allogenic cell therapy has been established and is well understood (2, 3). It is estimated that scale-up manufacturing solutions for cell therapies will need to generate batches of up to 2000 L (3) of product using single-use, disposable technology. Dependence on single-use formats limits the selection of upstream hardware that is currently available, however; downstream hardware are more limited because processing solutions that are currently available are typically incompatible with the unique requirements of cell-therapy products (4).

Yet, even if allogenic cell therapies lend themselves to a scale-up manufacturing strategy, there are limits to how scale up can be implemented for this type of therapy. Unlike mAb production—where the goal is to produce higher titers of cells containing the antibody—in cell-therapy, the cell itself is the therapeutic agent. The cells must, therefore, keep their integrity and remain functional, two aspects that are challenged when the cell density in the biomanufacturing environment increases. As the number of cells rise, the risk of introducing inhomogenity also increases; if inhomogeneity increases, then cellular performance can also be altered, impacting the quality and effectiveness of the cell therapy. By scaling up cell production, process developers and operators must also overcome the challenges of properly scaling other aspects of the manufacturing

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process, such as cell harvesting, downstream processing, and formulation technology, to ensure that the final cell therapy product meets quality standards and therapeutic requirements (2).

Autologous cell therapies, in comparison, do not lend themselves to a scale-up manufacturing strategy because each treatment is patient specific. Cells must first be collected from the patient, transported from patient to a manufacturing facility where the cells are processed, and then shipped back to the patient for administration. The entire manufacturing chain, from patient back to patient, must retain sterility and traceability. Thus, autologous cell therapies require a decentralized scaled-out manufacturing process—to produce a single batch of therapy for each patient. This poses a challenge to conserving cost making economies of scale difficult to achieve (3). Lowering the costs associated with commercial-scale autologous cell-therapy manufacture therefore relies on advanced engineering solutions and innovative manufacturing technologies that reduce the complexity and labor-intensive steps that currently exist in cell therapy manufacture (2).

For commercial autologous cell therapy production, a scale-out scenario would entail a complete manufacturing loop—from patient to manufacturing facility and back to patient—set up per patient. Challenges are largely rooted in the logistics of the workflow, such as the location of each patient, how close they are to a cell-therapy manufacturing facility, maintaining sterility and traceability throughout the entire loop, preserving cell integrity and functionality until the therapy is administered to the patient, and time constraints where cell viability is time dependent. Bioprocessing equipment and materials suppliers—as well as contract manufacturing organizations—are racing to develop suitable tools and processes to meet the manufacturing demands of these personalized treatments.

The gene therapy challenge

Gene therapy manufacturing has similarities to mAb manufacturing that allow for some steps in its process to be scaled up. Currently, viral vectors are a popular method used to deliver gene therapy. Mammalian cells are cultivated to produce the viral vector "package," and—as in cell culturing for mAbs—cell density can be increased by using larger-volume bioreactors. In the case of gene therapy, you may scale out by adding more bioreactors. The downstream purification process can be scaled up, as the purification process for a gene therapy can use similar hardware as is used in mAb purification.

"In general, purification processes in gene therapy are able to be scaled up to larger volumes because most of them

use standard types of biotechnology unit operations such as chromatography, membrane, and/or hollow fiber filtration. On the downstream purification side, much of the technologies one would use for gene therapy are commonly used on mAbs now," says Diane Blumenthal, head, technical operations, Spark Therapeutics. Spark received FDA approval for its gene therapy product, Luxturna (voretigene neparvovec-rzyl), for treating an inherited retinal disease, in December 2017 (5).

"For example, mAbs rely heavily on affinity resins, which are very specific to the mAb itself. In the case of gene therapy, the resins would be specific to the capsid protein. The downstream purification aspect lends itself nicely to standard purification technology. Although there are many technical biochemical nuances to take into consideration, the unit operations themselves are very similar to mAbs," Blumenthal adds.

For other steps of gene-therapy manufacturing, however, a scale-up strategy is more complex. One major challenge to a scale-up strategy lies in the crucial step of transient transfection, which is when DNA is introduced into the mammalian cell. The genetic material to be introduced into the mammalian cell include the DNA that encodes the cell's machinery to make the virus, which will be the vehicle through which the therapy is delivered, and the DNA that will be delivered to the patient's cells (i.e., the therapy). The virus packages the therapy DNA, making it ready for delivery (i.e., the viral vector).

The transient transfection process has not been perfected to be effective at significantly large volumes, and a significant barrier to that lies in the fact that the transfection process is a highly complex process, according to Blumenthal. There is a limitation on how much DNA each cell can take up. In addition, there are challenges posed by having high DNA densities present during transfection. DNA is sticky and especially likes to stick to itself and to cells. The more DNA there is in a mixture, the higher the chances that the transfection process will be less efficient.

"With a mAb, we can grow 10,000 L or more.

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Publication Comparing Cell Culture Methods

But, [for a gene therapy] we haven't quite perfected the ability to grow cells to a high cell density as we have been able to do with a mAb and still be able to conduct the transient transfection process. We haven't perfected the technology to get us to those large volumes where we can produce gene therapy product in larger amounts, quickly, while improving upon the cost of goods economics," Blumenthal states.

Based on the inherent limitations posed by the transient transfection process, a simple scale-up method may not work; a scale-out method may be more appropriate. That would entail making smaller cell culture batches and combining them together at an appropriate place in the production manufacturing process. "There needs to be a balance between how much DNA can be put into a concentrated volume, how high the cell density can be, and how capable the cell machinery is to take up the DNA and produce the required virus for packaging the therapeutic DNA component. Overall, it's a very complex biological process. We understand the process, but we don't yet understand well enough every little nuance nor the complex needs of the transient transfection process to be able to apply engineering principles to overcome the biochemistry, the virology, and the molecular biology that is associated with it," Blumenthal says.

In the specific case of Luxturna, Spark uses a roller bottle process in the cell culture step, an older technology still used to manufacture some vaccines. In the roller bottle process, cells grow and attach to the surface of cylindrical bottles and are bathed in growth media as the bottle is rolled on a rack inside a temperature-controlled chamber. To increase production volumes using a roller bottle process, a scale-out strategy could be used. Scale-up technology is also available for this type of culture methodology. They are not, however, more efficient than suspension culture methodology.

"On the upstream cell-culture side, Luxturna is made using an attachment-dependent system which, when you really want to do more volume, you do multiple weeks' worth of production at a certain scale and then we combine them together at the final step, after the product has been purified," Blumenthal elaborates.

Spark is, however, looking toward advancements in technology for their processes. For example, the company is moving almost all of its products to more standardized technology. In the case of the cell culture process, it is moving toward a suspension cell culture (i.e., bioreactor), so it would no longer need to rely on an attachment-dependent cell culture. "Using a bioreactor allows for us to scale up as well as scale out," Blumenthal says.

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Scale-Up of *Escherichia coli* Fermentation from Small Scale to Pilot Scale Using Eppendorf Fermentation Systems

Bin Li and Ma Sha

Abstract

The scale-up of fermentation processes is critical to the success of industrial fermentation for the production of biologicals in the biopharmaceutical market. Eppendorf bioprocess systems are available with autoclavable, single-use and sterilize-in-place vessels and together cover a wide range of working volumes from less than 1 L to as large as 2,400 L. In this application note, we used *E. coli* fermentation to demonstrate the scale-up capabilities of Eppendorf fermentation systems from small scale to bench scale and pilot scale.

To determine suitable parameters and setpoints for the operation of each fermentor, we considered critical scalability-related engineering parameters.

The parameters described include proportional vessel/impeller geometry, oxygen transfer rate (OTR), impeller power numbers (Np) and impeller power consumption per volume (P/V).

We carried out *E. coli* fermentation runs at three different scales (1 L, 10 L, and 100 L) following a constant P/V strategy, and represented the *E. coli* biomass growth trends by plotting optical density (at 600 nm, OD_{600}) curves over time. The fermentation runs at each of the three scales produced very similar biomass yields over time, indicating excellent scalability within the Eppendorf fermentor product family.

Introduction

The ultimate goal in bioprocess development is large-scale commercial production. Production-scale process optimization is usually cost prohibitive, so optimization is often performed in smaller scale bioreactors and fermentors. An optimized small-scale process can then be transferred to pilot

Figure 1: Eppendorf fermentation systems, from small to pilot scale, used in this study.

Small scale	Bench scale	Pilot scale
		
DASGIP® Parallel Bioreactor System with 4-fold Bioblock	BioFlo® 320 bioprocess control station	BioFlo 610 mobile pilot scale fermentor
Used with 1 L fermentation glass vessel	Used with 10 L stainless steel dished-bottom glass vessel	Used with 100 L sterilize-in-place (SIP) stainless-steel vessel

scale following established scale-up strategies.

The basics of successful fermentation scale-up start with proportional fermentor and impeller design. Eppendorf fermentation systems were designed following bioprocess industry stirred-tank design standards and provide excellent scalability from geometrical perspective. The intention of this study was to evaluate the scale-up of *E. coli* fermentation. To do so, we first investigated engineering parameters critical for scaling up fermentation processes, such as vessel and impeller geometry, oxygen transfer rate, power number, and impeller power consumption per volume. Then we utilized these data to scale-up an *E. coli* process from small scale (1 L) to pilot scale (100 L) following the constant P/V scale- up strategy.

Materials and Methods

Equipment

The Eppendorf fermentation systems, from small scale to pilot scale, used in this project are shown in **Figure 1**. Vessel parameters critical for scale-up are listed in **Table I**.

Table I: Proportionally designed vessels and impellers at different scales

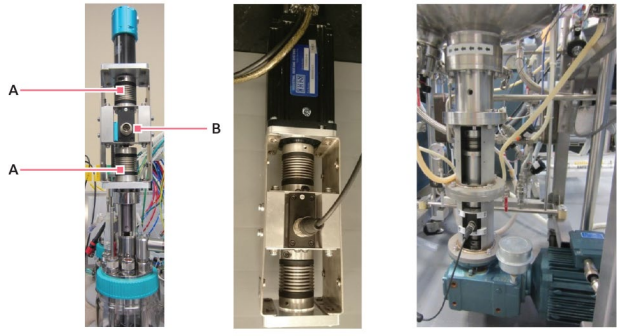
	Bioblock 1 L	BioFlo 320 10 L	BioFlo 610 100 L
Maximum gas flow (SLPM)	4.2	20.0	150.0
Vessel type	Glass	Glass	Stainless steel
Working volume (L)	0.2 – 1.0	3.5 – 10.5	32.0 – 100.0
V_{max} height (mm)*	136.0	323.0	904.0
Vessel inner diameter (ID) (mm)	100.0	211.0	381.0
Ratio V_{max} height : vessel ID per impeller	0.7	0.8	0.8
Impeller style; impeller material		Rushton/Rushton-type; 316 L	
Impeller quantity	2	2	3
Impeller diameter (D) (mm)	46.0	84.4	165.1
Ratio impeller diameter : vessel ID	0.4	0.4	0.4
Max. agitation (rpm)	1,600	1,200	500
Max. tip speed (m/s)	3.85	5.30	4.32

* V_{max} height = Height from bottom of the vessel to the top surface of the liquid at maximum vessel working volume

Table II: Correlation between tip speeds (m/s) and agitation speeds (rpm) for the three systems

Tip speed (m/s)	Agitation speed (rpm)		
	Bioblock 1 L	BioFlo 320 10 L	BioFlo 610 100 L
0.6	249	136	69
1.2	498	272	139
1.8	747	407	208
2.8	1,163	634	324
3.8	1,578	860	440

Figure 2: Torque sensor and adaptor setups used in DASGIP bioreactor (left), BioFlo 320 (middle), and BioFlo 610 (right): A: The torque sensor adaptor (two pieces), B: Torque sensor.



Investigation of engineering parameters

Oxygen transfer rate (OTR)

OTR is the rate at which oxygen is transferred from air to the liquid in a vessel, and OTR values provided by equipment manufacturers are often the maximum OTR. Since oxygen is often the limiting factor during aerobic fermentation, it is important to select equipment of different sizes with similar OTR capabilities so that the small-scale success can be replicated at the large scale. We conducted the OTR measurements using a previously published protocol based on sulfite depletion (1), with the exception of air flow, which was set to 1.5 vessel volumes per minute (VVM) to match the specification of the BioFlo® 610.

Power number (Np)

The (impeller) power number (also known as Newton number) is a dimensionless number associated with different type of impellers. Np is commonly used to calculate impeller power consumption during bioprocess scale-up (2). We determined the power number and P/V values at various tip speeds toward the higher agitation rates that are usually used for fermentation. Since the controllers display rpm rather than tip speed, a translation between tip speed and agitation rpm for each system is shown (**Table II**). One way to experimentally determine the Np is to measure impeller torque using a rotational torque sensor (**Figure 2**), and to calculate the power number using the following equation [2]:

$$\text{Power Number (Np)} = \frac{2\pi(M - M_d)}{\rho N^2 d^5}$$

M: Torque (with full working volume of DI water), (N·m)
M_d: Torque (empty vessel), (N·m)
ρ: DI water density = 1,000 kg/m^3
N: Agitation speed (rps)
d: Impeller outer diameter (m)

It is important to measure net impeller torque without bearing resistance. The impeller torque is equal to the torque value measured in deionized (DI) water at a given agitation rate and then subtracting the torque value measured in the empty vessel at the same agitation rate.

It is a common practice in scale-up studies to determine power numbers without gassing. However, gassing greatly reduces impeller torque, and thus has a significant impact on the apparent impeller power numbers as well as the results of aerobic fermentation. Since typical fermentation experiments are conducted under high gassing conditions, we have decided to obtain Np under a high gas flow of 1.5 VVM in addition to under "no gassing" conditions.

The purpose of determining Np is to calculate the impeller power consumption per liquid volume (P/V, W/m^3). P/V can be calculated from Np using the following equation [6]:

$$P/V = \frac{Np \times \rho \times N^3 d^5}{V}$$

ρ: DI water density = 1,000 kg/m^3
N: Agitation speed (rps)
d: Impeller outer diameter (m)
V: Full working volume (m^3)

Bioreactor setup and *E. coli* fermentation

Mini cell bank

We used *E. coli* (ATCC® 25922GFP™) in the fermentation runs of all three different scales. We aseptically thawed the stock vial into a 15 mL conical tube along with 5–6 mL of BD Bacto™ Tryptic Soy Broth (TSB) medium (Becton, Dickinson, USA). We spread 100–500 µL of TSB medium containing *E. coli* onto an agar plate and incubated it at 37°C overnight. We inoculated several 500 mL baffled shake flasks each containing 100 mL TSB medium from a single colony of the agar plate. The culture was grown in a New Brunswick™ Innova® 44 Shaker at 37°C, 200 rpm overnight and then centrifuged in an Eppendorf Centrifuge 5810 R at 4°C, 3,700 rpm for 10 min to collect the *E. coli* pellet. We used an equal volume of TSB medium containing 15% glycerol to suspend the cells, and the resulting *E. coli* suspensions were mixed and aliquoted into 1 mL tubes for storage at -80°C as mini cell bank.

Inoculum

We retrieved a frozen vial of *E. coli* from the mini cell bank and added 20 µL into 500 mL of TSB medium in each 2 L shake flask (VWR®, UK). We incubated the culture at 37°C, 200 rpm overnight in an Innova 44 shaker to form the *E. coli* inoculum stock. The number of shake

"It is a common practice in scale-up studies to determine power numbers without gassing."

flasks needed depended on the vessel sizes in the fermentor step. The OD_{600} values of the inocula were typically about 12.

Fermentation and feeding protocol

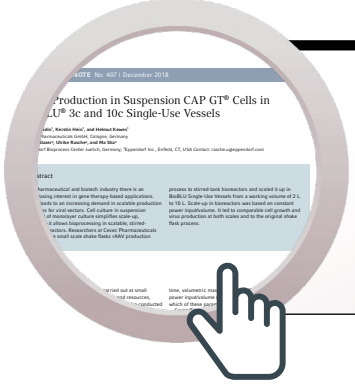
We chose to use 90% of the vessel maximum working volume for all three fermentors. *E. coli* was cultured in a chemically defined medium of pH 7.0. The initial fermentation medium we prepared as follows: 10% working volume of 10 x phosphate/citric acid buffer (133 g/L KH_2PO_4, 40 g/L $(NH_4)_2HPO_4$, 17 g/L citric acid) and 76.5% working volume of deionized water were added to the vessel for sterilization. After the medium was cooled to growth temperature or room temperature, we added the following sterile components aseptically to make the complete fermentation medium: 1% working volume of 240 g/L $MgSO_4$, 22.7 ppm of the working volume of 20 g/L thiamine, 1% working volume of 100 x trace element solution, 1.48% working volume of 70% glucose solution and 0.2% working volume of 500 x carbenicillin stock solution. The 100 x trace element solution contained: 10 g/L iron (III) citrate, 0.25 g/L $CoCl_2 \cdot 6H_2O$, 1.5 g/L $MnCl_2 \cdot 4H_2O$, 0.15 g/L $CuCl_2 \cdot 6H_2O$, 0.3 g/L H_3BO_3, 0.25 g/L $Na_2MoO_4 \cdot 2H_2O$, 1.3 g/L zinc acetate · $2H_2O$, and 0.84 g/L EDTA (3-4).

We separately prepared a concentrated feeding medium: 5% feeding medium volume of 240 g/L $MgSO_4$, 0.83% feeding medium volume of 20 g/L thiamine solution, and 15% feeding medium volume of 100 x trace element solution were mixed with 70% glucose solution to the final volumes of 0.2 L, 2 L, and 20 L for the three different scales.

To maintain a constant working volume throughout the fermentation process, a continuous fermentation method was used, and volumes of *E. coli* culture identical to the volume of feeding medium added were removed upon feeding. The feeding-in and pumping-out protocol shown in **Table III** illustrates the adjustments made to the pump speed over the course of the fermentation.

In all three fermentors, we inoculated the growth medium with an inoculum volume of 10% of the initial fermentation medium volume. Antifoam 204 (Sigma-Aldrich®, USA) was added only when foaming was observed.

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Table III: Proportional feeding and broth removal strategy for continuous fermentation runs at different scales

Time (h)		3.5	4.5	5.5	6.5	7	7.5	8	8.5	10
Pump speed (mL/min)	Bioblock 1 L	0.07	0.13	0.2	0.39	0.53	0.67	0.88	1.03	1.7
	BioFlo 320 10 L	0.7	1.3	2.0	3.9	5.3	6.7	8.8	10.3	17
	BioFlo 610 100 L	7	13	20	39	53	67	88	103	170

We monitored cell growth offline using samples taken every hour.

pH calibration and control

We calibrated the pH sensors outside the vessels prior to autoclaving them, using a two-point calibration method and standard buffers. We used the buffer of pH 7.0 to set "ZERO" and the buffer of pH 4.0 for the "SPAN"/"slope" (please refer to the DASware® control and BioFlo user manuals). The pH was automatically maintained at 7.0 by adding 25% (v/v) NH_4OH via a pump (assigned as "base"). The deadband for pH control was set to 0.05.

Dissolved oxygen (DO) sensor calibration and gassing control

We performed an analog polarographic DO sensor calibration using a standard two-point calibration method:

0% (set "ZERO") was obtained by disconnecting the sensor from the cabinet and allowing the raw value to stabilize; 100% (set "SPAN") was obtained by running different agitation speeds for DASGIP 1 L, BioFlo 320 10 L, and BioFlo 610 100 L runs (822, 600, and 433 rpm, respectively) and 1.5 VVM (1.35, 13.5, and 135 SLPM, respectively) air flow until the DO value stabilized at the maximum.

Results and Discussion

As shown in **Table I**, all three Eppendorf fermentation systems from small scale to pilot scale were designed following the same vessel and impeller geometrical principles, which laid a good foundation for successful fermentation scale-up. However, it is also important to select equipment of different sizes with high OTR capabilities so that the different fermentation

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Webinar about Cell Culture Scale Up

Table IV: OTR measurements from DASGIP Bioblock to BioFlo

	Bioblock 1 L	BioFlo 320 10 L	BioFlo 610 100 L
OTR (mmol O_2/L/h)	392.6	486.7	349.3
Experimental conditions			
Air flow (SLPM/ VVM)	1.5/1.5	15.75/1.5	150.0/1.5
Agitation (rpm)	1,530	1,200	500
Tip speed (m/s)	3.68	5.30	4.32
Temperature (°C)	30	30	30
Liquid	DI water	DI water	DI water

scales can match each other in top line performance, and the small-scale success can be replicated in large scale. As shown in **Table 4**, all three fermentation systems achieved high levels of OTR, ~350 mmol/L/h or higher. This allowed scale-up fermentation runs to be carried out at high capacities, delivering matching biomass growth curves at very high bacterial densities.

Scalable geometry and matching high OTR provide the foundation and the framework for high-density fermentation scale-up experiments, but they did not constitute the scalability strategy in itself. Various strategies have been used for fermentation scale-up including constant tip speed, but the most reliable method to date has been constant power (P/V). It requires the determination of impeller power numbers (Np). We calculated Np numbers at different tip speeds up to 3.8 m/s (limited by the maximum tip speed of the small-scale system) following direct torque measurements

Figure 3: Power numbers converted from impeller torque measured from DASGIP Bioblock 1 L, BioFlo 320 10 L, and BioFlo 610 100 L vessels under "no gassing" (A) and "high gassing" (B) condition. The average was plotted based on triplicate measurements. The power numbers were obtained with factory default impeller combinations (dual Rushton/ Rushton-type impellers for DASGIP Bioblock 1 L, BioFlo 320 10 L and three Rushton-type impellers for BioFlo 610). Adding/reducing impellers or changing impeller design will affect power numbers.

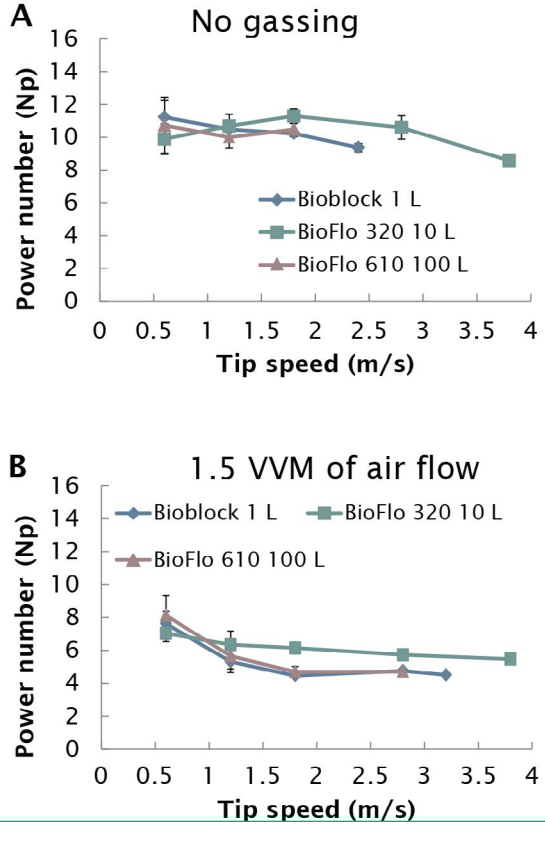


Table V: Average impeller Np values calculated from the results shown in Figure 3. The Np values at a tip speed of 0.6 m/s were not included in the calculation of the average

	Average Np without gassing	Average Np with 1.5 VVM of air flow
Bioblock 1 L	10.0	4.6
BioFlo 320 10 L	10.3	5.9
BioFlo 610 100 L	10.2	5.0

described in the Materials and Methods section (**Figure 3**). Np is a constant under turbulent conditions [5]. Under less turbulent conditions such as a lower tip speed of 0.6 m/s, the Np numbers may be slightly different than at higher tip speeds. Although the actual Np numbers varied slightly at different tip speeds (as shown in **Figure 3**), they were very similar to each other and the average could be considered a constant in guiding fermentation scale-up. The impeller Np for Eppendorf fermentation vessels are ~10 without gassing and ~5 with 1.5 VVM of air sparging (**Table 5**).

From the measured Np numbers, we calculated the impeller power consumption per liquid volume (P/V, W/m3) as described in the Material and Methods section (**Figure 4**). Maintaining constant P/V between vessels is one of the most accepted strategies for scale-up.

To design high-density *E. coli* fermentation scale-up, the Np values obtained under 1.5 VVM air flow (**Figure 4B**) were used. We curve fit the data points and added trend lines (**Figure 5**). The maximum P/V achievable by all three scales was ~2.42 kW/m^3, which wes elected to be the constant P/V value governing the fermentation scale-up (**Figure 5**). Back-calculating the agitations from this P/V value determined that 822, 600, and 433 rpm were the agitation values to be used for Bioblock 1 L, BioFlo 320 10 L, and BioFlo 610 100 L, respectively.

Figure 4: P/V values calculated based on Np values from Table V.

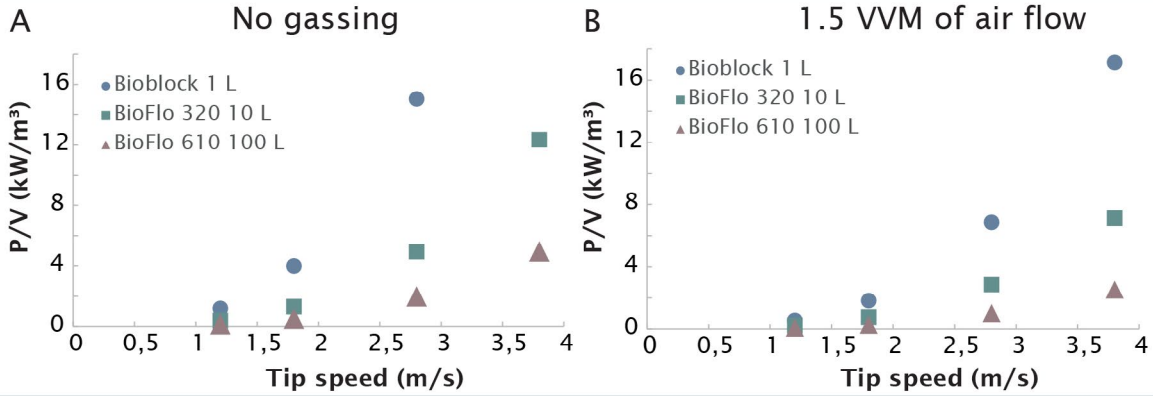


Figure 5: Determining the constant P/V values for scale-up under 1.5 VVM of air flow

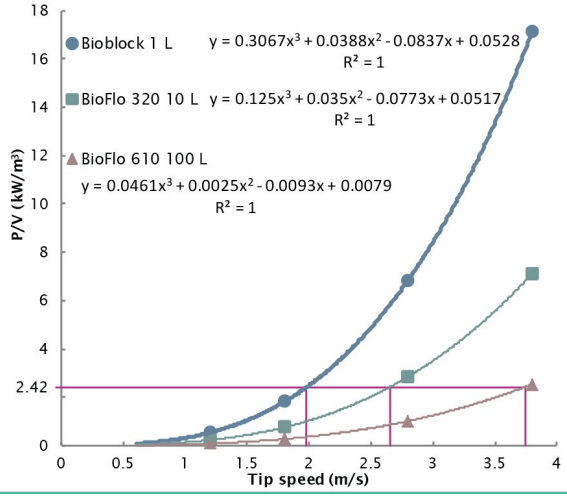
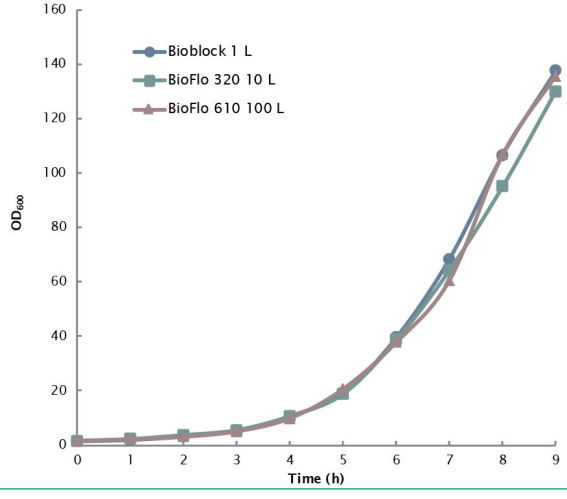


Figure 6: Fermentation biomass growth curves among all three systems. Fermentations were carried out using a constant P/V-value of 2.42 kW/m³, which was determined from Fig. 5



Furthermore, all three fermentation runs were conducted under 1.5 VVM of constant air flow.

We conducted three fermentation runs and took samples hourly to monitor the cell growth (OD_{600} value) as described above. As shown in **Figure 6**, the growth curves from all three fermentation runs produced very similar profiles, indicating that excellent scalability has been achieved using the constant P/V scale-up strategy.

Conclusions

The Eppendorf fermentors from Bioblock to BioFlo are of geometrically and proportionally similar stirred-tank design. All three systems are capable of delivering high OTR values, providing excellent capability for high density aerobic fermentation in a scalable manner. Maintaining constant P/V between different vessel sizes in fermentation scale-up from Bioblock 1 L to BioFlo 320 10 L to BioFlo 610 100 L produced nearly identical *E. coli* growth curves, providing solid proof for the scalability of Eppendorf fermentation systems. All three fermentation systems were able to support high-density *E. coli* growth at or near 140 OD_{600} within 9 hours of inoculation. In addition, the Np values of the impellers can be used for further scale-up or scale-down studies between Eppendorf and the stirred-tank fermentation vessels of other manufacturers.

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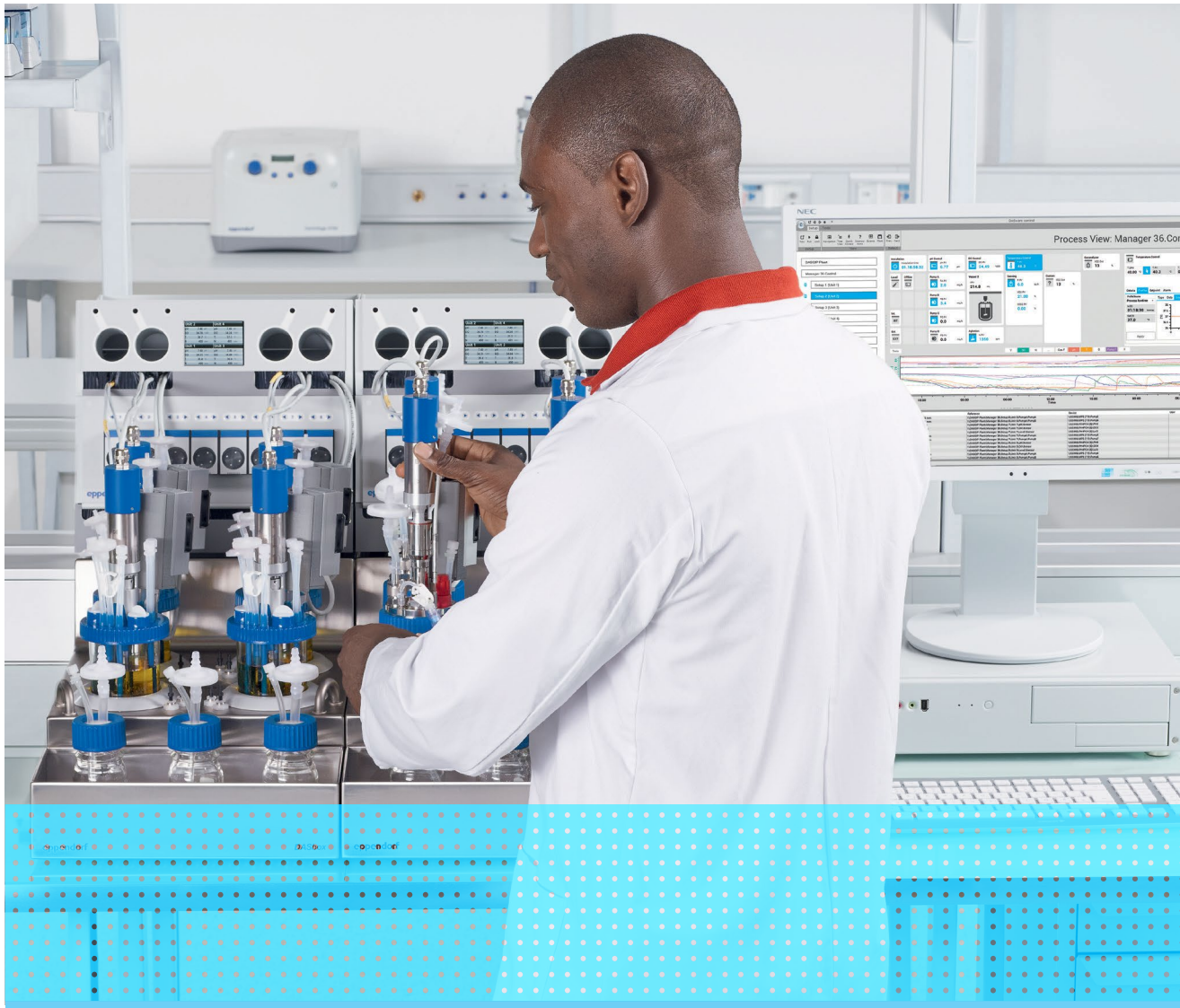
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Bioprocessing Scale-Up Challenges and Eppendorf's Newest Solution: Thought Leader Q&A

Interview with Ma Sha

Eppendorf's latest bioprocessing scale-up offering, a Pilot Scale Cell Culture SUB controller, uses innovative technology to deliver ease of use in addition to valuable time and cost savings.

During bioprocess scale-up, tremendous effort goes into matching yields between small and large scale. *BioPharm International* recently sat down with Ma Sha, PhD, Head of Bioprocess Applications at Eppendorf, to discuss current gold standard bioprocess scale-up strategies. Topics included the shift from the constant kLa technique to the constant power over volume (P/V) method and state-of-the-art features of Eppendorf's new BioFlo 720 bioprocess control system.

BioPharm International: How have bioprocessing scale-up challenges changed over the years? What hurdles do clients face during scale-up?

Sha: Several main scale-up strategies have been used over the years, some of which were used in the chemical engineering/mixing industry before the bioprocess market was started. The gold standard scale-up strategy has been constant volumetric mass transfer coefficient (kLa), which allows biomass and production yield to be kept constant throughout scale-up. If everything remains optimized between different-sized vessels, there will be an uptake of oxygen to the cells, supporting proportional cell growth and protein production.

In recent years, the gold standard scale-up strategy has switched from using constant kLa to using constant P/V, a method that we have already been using for approximately five years. Although we still produce kLa numbers with our scale-up studies to support our customers, Eppendorf does not actually use constant kLa for scale-up anymore

because it is much less efficient than the constant P/V method, requiring the measurement of a lot of kLa. The kLa value changes with just about every parameter—different volumes, agitation speeds, and gassing rate etc.—whereas the constant P/V method requires only a single constant to be measured: the Power Number (Np). It has been shown that constant P/V can deliver constant kLa, thus achieving the same scale-up results with much less effort. The power number is used to calculate P/V, and it drastically reduces the amount of upfront efforts needed to achieve matching yields between scales.

A final point to mention is that the concept of using constant power with scale-up is simple—but with the assumption that the power numbers have been validated. There are different ways to obtain power numbers, however they will not necessarily match unless they have been validated. A user may try to scale up between different manufacturers vessels using power numbers, but it can be challenging when they were obtained from different sources.

BioPharm International: How does the constant power strategy ensure successful scale-up?

Sha: From a strategic perspective, constant power can be thought of as an energy conservation method. Constant power over volume is essentially the power input and is a form of energy. The output is a biomass, which could be considered another form of energy. If the system is equally efficient among different scales, then energy is conserved (i.e., the power input translates into proportional biomass).

But, it's not a magic bullet. Constant power alone has limitations when trying to perform scale-up with too big of a leap. For example, when trying to scale up from 20 liters to 2,000 liters, constant power by itself may not be sufficient. Other things can be brought in to compensate such as maintaining constant dissolved carbon dioxide levels (dCO_2%). Studies show that when constant dCO_2% was added where the constant P/V scale-up was a bit off, the yield match was achieved over a fairly large vessel size difference.

BioPharm International: What other scale-up strategies have you

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Panel Discussion: Streamlining Biologics Scale-Up

seen in terms of technology to support streamlined scale-up?

Sha: The goals for scale-up generally involve optimizing yield on the smaller scale to save money. Developers want to carry that greater yield up to a larger scale.

Because P/V scale-up is still quite challenging and requires significant expertise, smaller companies might not have the resources to do it. They might use an easier, but less accurate approach. For example, constant tip speed is sometimes used as a substitute. It provides constant shear force to the cells, which means that during scale-up, cells are not damaged any more on the larger scale than they would be on the smaller scale. Constant tip speed does not necessarily result in perfect yield match, however. There may be some loss in yield as the scale is increased. Nonetheless, constant tip speed method enables them to move their development forward faster.

Meanwhile, power number and constant power have become a standardized approach for larger companies. They often have personnel dedicated just to performing Np measurements and P/V calculations. These parameters are then used for their pilot-scale run.

BioPharm International: How can Eppendorf help in the latest bioprocessing product offering?

Sha: Eppendorf decided to bring its scale-up expertise into the new BioFlo 720 bioprocess control system using a software innovation called Scale-Up Assist. The BioFlo 720 internally calculates constant power, ranging from Eppendorf's BioBLU vessels on the small and bench scale all the way up to the 2,000 L pilot-scale Single-Use Bioreactors. A customer can enter small-run parameters, and the BioFlo 720 will predict the large-run parameters automatically to obtain matching biomass and protein production yield.

BioPharm International: In addition to the Scale-Up Assist, are there any other features of the BioFlo 720 that you can highlight?

Sha: Several features were included in the BioFlo 720 with usability in mind. One nice feature introduced is the compatibility with many different types of dissolved oxygen (DO) sensors and the new auto calibration feature. In addition to the traditional polarographic DO sensor, Eppendorf can provide the customer with digital and optical DO sensors. The BioFlo 720 includes three different types of DO sensors: the traditional polarographic DO, the digital polarographic DO and the digital optical DO. The calibration process is not always the same among them, so to avoid customers getting confused, we included an innovative feature in BioFlo 720 called Auto Calibrate. Regardless of which

DO sensor is plugged in, the BioFlo 720 controller will recognize it and automatically execute the calibration. This will be a significant user advantage, eliminating the learning curve for users resulting in more time to focus on the process.

In addition, the BioFlo 720 is the first Eppendorf controller to use Thermo Fisher Scientific's pilot-scale cell culture Single-Use Bioreactor (SUB). So, the BioFlo 720 was designed with an Auto Inflate function that is helpful for inflating very thin, plastic single-use bags, which is typically a precarious task. Unlike BioBLU vessels, which have a hard shell, single-use bags are not very sturdy and can burst easily. The Auto Inflate feature was developed to prevent this and ensures a safe and reliable inflation of the single-use bags. The BioFlo automatically recognizes the connected bag and inflates it with the correct amount of gas. It thereby removes the risk of manual operational errors and saves time. The user can just hit the button and walk away instead of having to manually inflate and be there the whole time. Also of note, Eppendorf's BioBLU vessels can be extended from 50 L to 2,000 L with Thermo Fisher's single-use solutions.

We believe that these features combined help to make processing scale-up as streamlined and efficient as possible, particularly for smaller companies.





From Shaker to Pilot/ Production Bioreactor: How Scale Up Assist Using the BioFlo® 720 Bioreactor Control System Can Help Your Antibody Production Workflow

Amanda Suttle, John Longsworth, and Ma Sha

Scale Up Assist decreases process development time while ensuring consistent mAb yields.

Abstract

To scale upstream bioprocesses, an effective scale-up strategy is required to ensure the reproducibility of both cell culture growth and batch yields at large working volumes, ideally with little or no additional optimization. The Constant Power approach is a successful method for matching bioreactor yields at different scales. This study details the use of the new Scale Up Assist feature of the BioFlo 720 Bioreactor Control System to scale CHO batch cultures from 3 L to 10 L BioBLU® Single-Use Vessels and from the 10 L to 50 L Single Use Bioreactor (SUB) bag. Using a built-in constant power software algorithm, the Scale Up Assist specified what agitation and gassing set points to use for different vessel sizes.

We cultivated Chinese Hamster Ovary cells (CHO) using BioBLU Single-Use Vessels and a Thermo Scientific™ HyPerforma™ 50 L SUB. Our results demonstrated that the parameters provided by the new Scale Up Assist software resulted in similar cell growth and monoclonal antibody (mAb) production yields for all three vessel sizes. Adoption of the controller's Scale Up Assist function can decrease process development time while also helping to ensure consistent mAb yields across vessel sizes and bioreactor platforms.

Introduction

Bioprocess development is often done at small or bench scale to save production costs, optimize ideal conditions for cultures, and shorten time to market. An effective strategy is required during scale-up to ensure matching cell growth in cultures and similar mAb or other protein production yields from

small, bench, and pilot scale. However, sometimes these strategies to scale-up cell culture processes are not as straightforward as we would like. Many strategies have been established to streamline the scale-up of protein and mAb production. These strategies include keeping a constant tip speed across bioreactors, matching oxygen volumetric mass transfer coefficients (K_La), and maintaining constant impeller power per unit volume (P/V). We have utilized the Constant Power approach, manually calculated, for scale-up in both fermentation [1] and cell culture [10] applications. This method involves calculating impeller power consumption per unit volume using Equation 1:

$$P/V = Np \times \rho \times N^3 \times d \quad \text{(Equation 1)}$$

Np is the dimensionless impeller power number or Newton number [2], ρ is the density of water (1000 kg/m^3), N is the impeller speed (rpm), d the impeller outer diameter (m), and V is the working volume of the culture (m^3). This strategy has proven to be an effective method to maintain similar bioreactor yields from bench to pilot scale. Our goal was to test the constant P/V based on the Scale Up Assist feature to automatically match mAb production yields from our CHO culture using BioFlo 320 and BioFlo 720 bioreactor control systems.

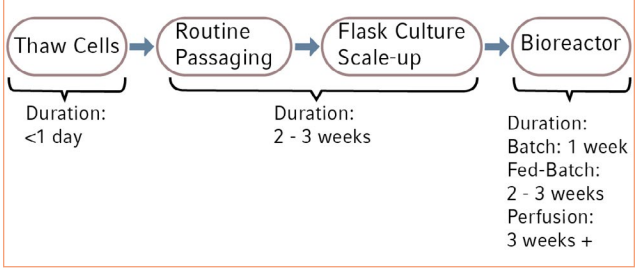
The BioFlo 720 is a new bioprocess controller that is compatible with Thermo Scientific's existing line of single-use bioreactors (SUBs). This controller introduces an innovative new feature termed Scale Up Assist, a built-in software algorithm that automatically calculates controller setpoints, greatly simplifying the process of scaling-up bioreactor mAb production processes. We employed the new Scale Up Assist feature to successfully scale our CHO culture from 3 L to 10 L BioBLU vessels and from 10 L to 50 L SUB.

However, no success in bioreactor scale-up can be achieved without a healthy and robust inoculum. We used the Eppendorf New Brunswick™ S41i CO_2 Incubator Shaker to prepare the initial suspension CHO inoculum, and seeded our bioreactor with log phase cells at the highest quality level, >98.9 % viability. Furthermore, no cost-effective mAb production can be achieved without an economical way for medium preparation. We used a novel method consisting of a modified BioBLU 50c Single-Use Vessel, which yielded substantial savings in both costs and resources such as additional equipment needs.

Material and Methods

In these experiments, we used the BioFlo 720 Scale Up Assist to calculate the process parameters needed to scale up from 3 L to 10 L and from 10 L to 50 L. We cultured our CHO cells using

Figure 1: Overview of a typical cell culture mAb production workflow.



> "A critical aspect to designing any cell culture bioprocess, especially regarding scale up, is to achieve matching growth curves and production yields from shaker to pilot scale."

the BioFlo 320 and BioFlo 720 bioreactor control systems.

mAb production workflow overview

Our workflow begins when we thaw our cells. This can typically take less than a day between flask and media preparations to introducing the cells into their new their new environment in the incubator. We then move ahead to routine passaging and seed train or flask culture scale up. This step in our workflow can typically take anywhere from 2 to 3 weeks of culturing depending on the inoculation density we are targeting (**Figure 1**). Lastly, we focus on our bioreactor step. The bioreactor phase of our workflow can vary depending on our bioprocess procedure. For example, our batch run typically lasts a week, our fed-batch runs can last 2 to 3 weeks and our perfusion can be three or more weeks long. For these experiments, we chose batch runs. Now that our cell culture bioprocess workflow has been defined, we will break down each step further for each batch run in our experiments.

Thawing cells: inoculum

For all experiments, we used a proprietary suspension CHO cell line producing a human monoclonal antibody (hmAb) from TPG Biologics, Inc., cultivated in Dynamis™ AGT™ Medium (Thermo Fisher Scientific®). The medium was supplemented with 8 mM L-glutamine, 1% Antibiotic Antimycotic solution, and 1% Gibco® Anti-Clumping Agent (Thermo Fisher Scientific) for a complete medium [3]. We prepared our cell culture inoculum by cultivating our cells in single-use, baffled bottom shake flasks (Corning®) with a 20% maximum fill volume. We first thawed our cells from a previously cryopreserved vial and inoculated them into a 125 mL flask at a seeding density of 3×10^5 cells/mL. After our shake flask was seeded, we placed it into our New Brunswick S41i CO_2 Incubator Shaker. We set our shaking speed to 125 rpm and our CO_2 to 8%.

Figure 2: New Brunswick S41i CO_2 Incubator Shaker.



A critical aspect to designing any cell culture bioprocess, especially regarding scale up, is to achieve matching growth curves and production yields from shaker to pilot scale. Reducing shock factors such as temperature fluctuations can greatly reduce negative impact on your culture. The New Brunswick S41i CO_2 Incubator Shaker combines precise temperature and CO_2 control with one of the best laboratory shakers available, resulting in high cell yields and viability (**Figure 2**). We have found the S41i incubator shaker to be an optimal tool for achieving the perfect inoculum. An important feature of the S41i is the high temperature disinfection option. With a few simple clicks, your shaking incubator is clean and ready to go for your next bioprocessing challenge!

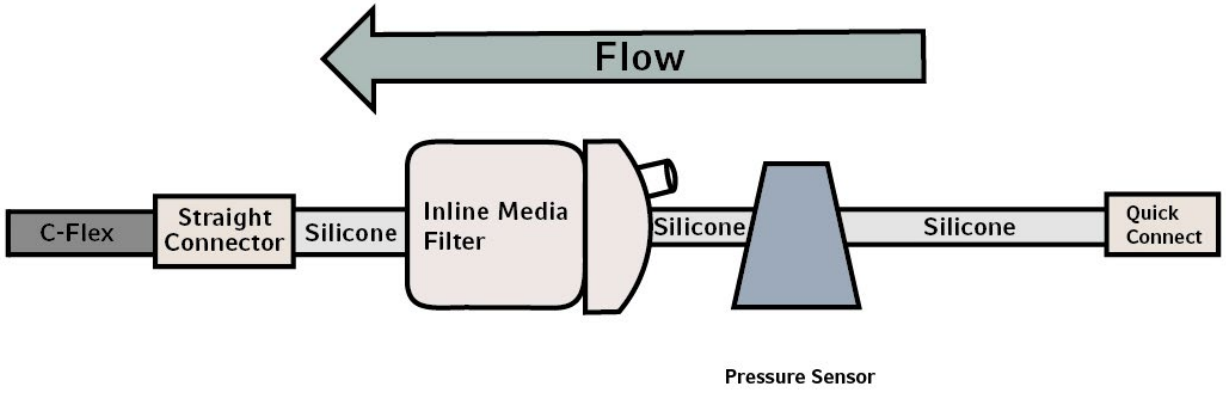
Figure 3: BioBLU 50c Single-Use Vessel connected to BioFlo 320 bioprocess controller.



Medium preparations for the 40 L run

Dynamis AGT Medium is a powdered medium that needs to be rehydrated and sterilized before cell culture use. To mix our medium thoroughly, we modified a BioBLU 50c Single-Use Vessel by drilling a large orifice near the top of the vessel. This allowed us to pour in our powdered medium and supplements into the vessel using a funnel (**Figure 3**). We rehydrated our medium, following the manufacturer's recommendations. First, we measured out 90% of the total volume of deionized (DI) water needed into our modified BioBLU 50c vessel. We then connected the BioBLU 50c vessel to our BioFlo 320 controller and started the agitation at 130 rpm. For every 1 L of medium prepared, 24.8 g/L of Dynamis AGT powder is needed. We calculated and weighed out

Figure 4: Media filtration setup.

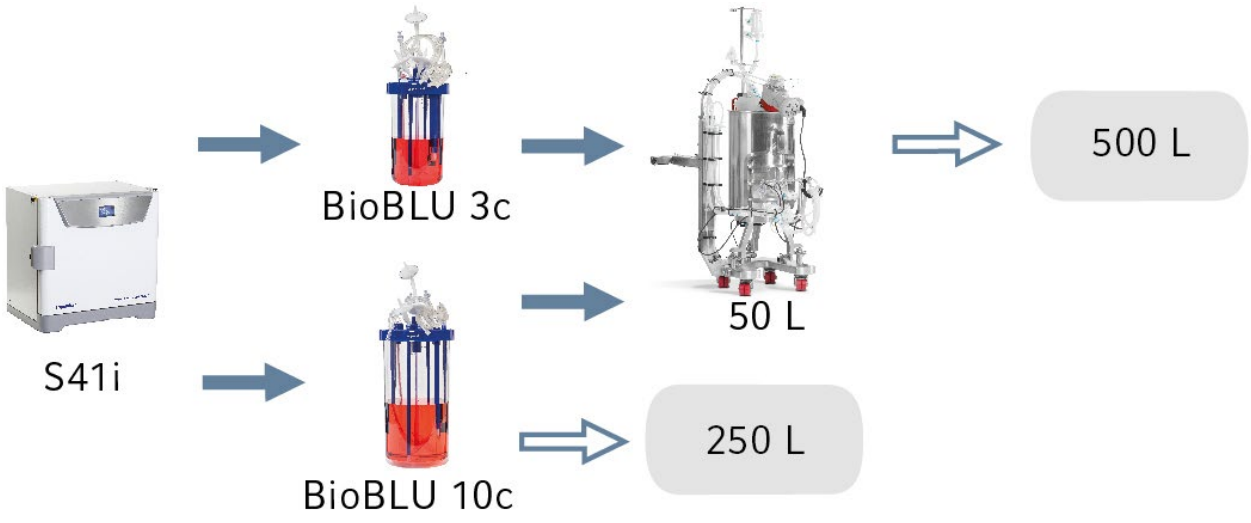


the amount of Dynamis AGT needed for a total volume of 40 L, taking into consideration the other supplements that are added to complete our medium. We then carefully poured that amount into our modified BioBLU 50c vessel using a funnel. After we added all the powder, we supplemented it with 8 mM L-glutamine, 1% Antibiotic Antimycotic solution and 1% Gibco Anti-Clumping Agent to complete the medium. We finally adjusted the volume with DI water to a volume of 40 L. We allowed the medium to mix for 30 minutes, as specified by the manufacturer, before connecting our filtration setup to our BioBLU 50c vessel. We connected our filtration setup by inserting the quick connect end to our harvest line on our 50c vessel (**Figure 4**). We sterilely welded the other side of our filter setup to our 50 L SUB using Cytiva's tube fuser-dry welder. We then placed the tubing from our set up that was before the filter into our 520U/R pump (Watson-Marlow®). We set our pump speed at 0.2 L/min to avoid rupture of our filter or tubing and to eliminate bubbles from the tubing line. We slowly ramped up our speed once our media had gone through our entire filtration setup and into our 50 L SUB. Our maximum pump speed was ca. 1.2 L/min, until we had roughly 5 L left of media to filter. We then ramped down our pump speed to around 0.2 L/min until the remainder of the media was filtered.

Routine passaging and seed train/flask culture scale-up workflow

After the initial thaw, we allowed our cells to grow for a few passages before scale-up in order to acclimate them to their environment. We determined that under our conditions, the optimal passage schedule is every other day. After monitoring our cell growth and viability (determined to be >95%), we increased our culture volume from

Figure 5: Seed train strategy for larger vessels.



125 mL to 250 mL, and finally, to 1 L shake flasks. During this scale-up, flask inoculation density, percentage fill and other parameters remained the same. Using this culture method, each bioreactor in this experiment was inoculated with cells that were approximately at the same passage and duration of the culture post-thaw [3]. For each experiment, the number of flasks per scale-up step varied, based on the amount required for optimal target inoculation and working volume of each bioreactor. For all experiments, our target inoculation density was 5×10^5 cells/mL. We also prepared additional extra flasks in case of suboptimal performance. After enough cells were obtained, we took the amount of culture that was needed from our 1 L flasks and combined them into a 2 L addition bottle (Eppendorf) that was prepared and autoclaved. We used this bottle method to inoculate our BioBLU 3c and 10c Single-Use Vessels.

For our 50 L SUB run, we needed a larger volume of inoculum. To reach this goal, we inoculated a BioBLU 10c Single-Use Vessel with a working volume of 10 L. Once growth in our bioreactor reached approximately 2×10^6 cells/ mL, we then welded our harvest line on to our BioBLU 10c and inoculated our 50 L SUB. Our scale-up seed train strategy used for the 40 L run can be applied to larger vessel sizes. By seeding a BioBLU 10c vessel and allowing culture density to reach approximately 2×10^6 cells/ mL, we were able to inoculate our 50 L SUB, with a working volume of 40 L, at 0.5×10^6 cells/mL. Using this approach, we could inoculate the 250 L and the 500 L vessels in a similar seed train

process flow (**Figure 5**). For example, to inoculate a 500 L run, we would prepare our initial culture in our S41i shaker. We would then scale-up our flask culture to inoculate a BioBLU 10c vessel. We would allow our culture to grow for a few days to achieve the calculated density needed to inoculate a 50 L SUB. We could then incorporate our 50 L SUB culture into our seed train by allowing the culture to grow for an additional few days to reach a high enough density to inoculate our 500 L SUB run.

Vessels

The BioBLU 3c and 10c Single-Use Vessels were operated by a BioFlo 320 bioprocess controller. Our BioFlo 320 was also used at the 10 L-scale to prepare the amount of inoculum needed for the 40 L culture. The BioFlo 320 controller was set up according to the manual [4]. A Thermo Scientific HyPerforma 50 L Outer Support Container was used for the 40 L CHO culture and was controlled by the BioFlo 720 bioprocess control system. A LAUDA® VC1200 water-cooled temperature control unit (TCU) was connected to the support container to provide heating to the jacket. We used a Thermo Scientific HyPerforma 50 L Bioprocess Container (BPC) with a 5:1 turndown ratio and drilled hole sparger (DHS) for our 40 L CHO culture. Our internal BPC pressure was measured using a PendoTECH® Single Use Pressure Sensor™ and monitored by our BioFlo 720 bioprocess controller.

40 L vessel preparation and bag inflation

The Thermo Scientific BPC was inflated using the BioFlo 720 Auto Inflate feature. The auto-inflation function automatically introduces a specified amount of air into the BPC until it is fully inflated. The auto-inflate program also has a built-in safety feature that will automatically shut down all gases in the event of high pressure being detected in the bag. Our pressure sensors were connected before inserting the bag into the outer support container by using the aseptic pressure sensor connector (Thermo Scientific).

Media filling process

Sterile medium was pumped into the BioBLU 3c and 10c vessels directly through a top addition line by welding onto the addition line with a Terumo tube welder and using one of the pumps located on the BioFlo 320 control station. Prepared medium was pumped into the 50 L BPC through a top addition line using the 520R2 pump head integrated on the BioFlo 720 cabinet. The medium for our 40 L run was sterilized by pumping it through Polycap TC filters after being prepared as described above. After 40 L was pumped into the BPC, we switched on the temperature

TABLE 1: Overview of process configurations and setpoints for all cell culture runs

Parameters	3c Setpoints	10c Setpoints	50 L Setpoints
Controller	BioFlo 320	BioFlo 320	BioFlo 720
Working volume	3 L	10 L	40 L
Agitation	151 rpm	126 rpm	169 rpm
Temperature	37 °C		
DO Sensor	polarographic sensor		
DO Setpoint	50 %, (P= 2; I= 0.09)	50 %, (P= 2; I= 0.09)	50 %, (P= 2.5; I= 0.09)
pH Sensor	potentiometric sensor		
pH Setpoint	7.0 (deadband = 0.1), cascade to CO_2 (acid), cascade to 0.45 M sodium bicarbonate (base)		
Target Inoculation Density	0.5 x 10^6 cells/ mL		
Gas strategy	3 – gas auto mixing		
Gassing range	0.04 SLPM – 1 SLPM	0.04 SLPM – 1 SLPM	0.04 SLPM – 13.33 SLPM

loop to warm the medium at 37° C for a minimum of 12 hours prior to inoculation.

Process parameters for all runs

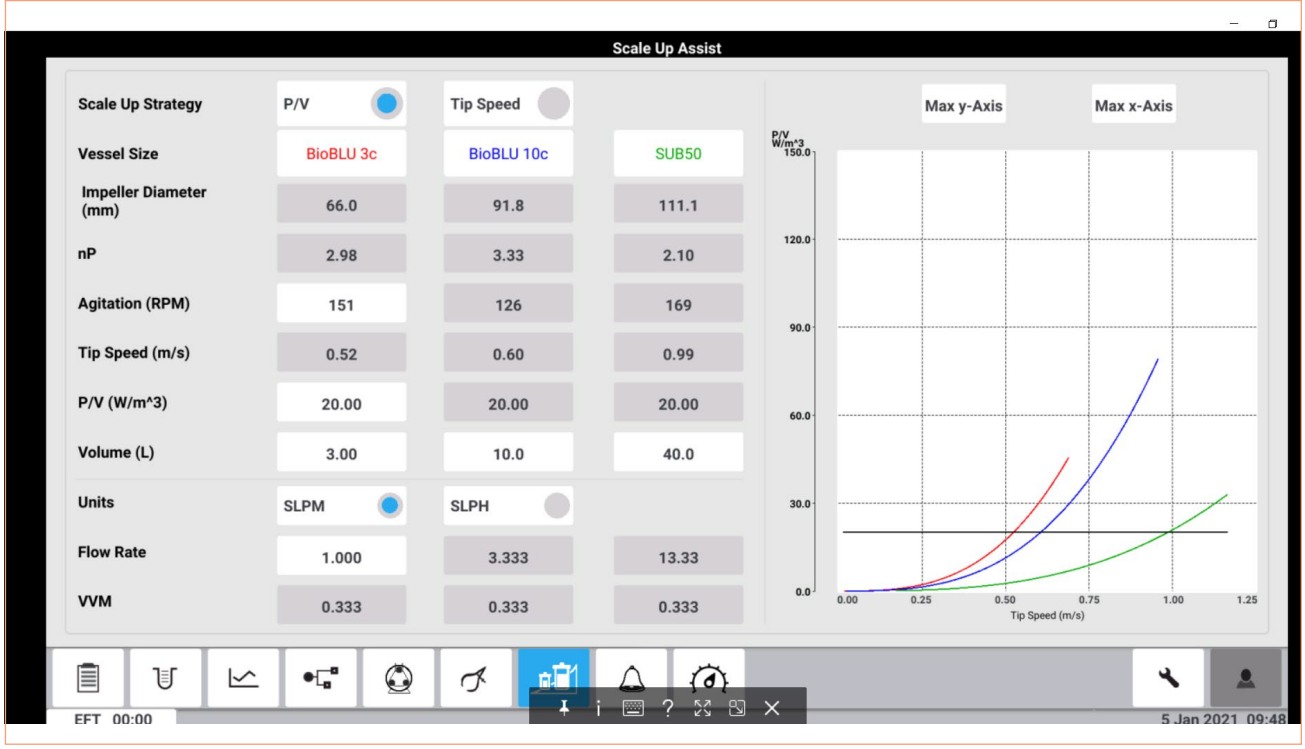
For all cultures, we measured dissolved oxygen (DO) by using Mettler Toledo® ISM® DO sensors. Our DO setpoint was set to 50% for all experiments and was controlled at setpoint using 3-gas auto mixing (**Table 1**). The P and I values selected for these experiments were optimized by using a dissolved oxygen simulation as previously described [8]. We used an analog pH sensor for all our experiments. Our pH setpoint was set to 7.0 with a deadband of 0.1 and was controlled via a cascade to CO_2 (acid) and 0.45 M sodium bicarbonate (base). For our 3 L culture, we sterilized our pH sensor after calibration in a sterilizing pouch and inserted the sensor aseptically

Figure 6: The BioFlo 720 bioprocess control system with 250 L SUB.



into a spare Pg 13.5 port on the BioBLU 3c vessel in our biosafety cabinet. Our pH sensor was calibrated based on the protocol in the BioFlo 320 manual [5]. For the 50 L culture, all sensors were inserted into probe assembly sleeves (Thermo Scientific) and autoclaved. The sensors were then inserted into the BPCs via the front sensor quick connectors, after inflation but prior to liquid filling. DO sensors were calibrated as previously described [6]. Dissolved CO_2 was monitored using a Mettler ISM CO_2 sensor that was also aseptically inserted in the Biosafety cabinet into a spare port on the BioBLU vessels after being sterilized separately. For our 40 L run, the sensor was sterilized using the SUB probe assembly and aseptically inserted into the SUB. For all experiments, temperature was controlled at 37° C and remained constant for the duration of the runs.

Figure 7: BioFlo 720 Scale Up Assist Screen.



Scale Up Assist feature

The BioFlo 720 bioreactor control system was designed to save time and to mitigate risks. (**Figure 6**). The well-known BioFlo software has been enriched with a variety of new features to automate the workflow, saving time, and simplifying repetitive tasks, such as the Scale Up Assist. Whether scale-up strategy is based on constant P/V or constant tip speed, the Scale Up Assist calculates all critical parameters needed, including gas flow rates and agitation with vessel specific data automatically populated in the software. Critical parameters for our experiments, including agitation and gas flow rates, were calculated by the new Scale Up Assist function on the BioFlo 720 (**Figure 7**). We used a constant power/volume (P/V) ratio of 20 for our scale-up calculations. Using this P/V ensured all bioreactors could be operated at reasonable tip speeds for their vessel size and geometry. The power numbers used for these calculations were determined experimentally for our BioBLU vessels in the Eppendorf applications laboratory and were provided by Thermo Scientific for the 50 L BPC [7]. These values have been programmed into the BioFlo 720 and are automatically activated when the corresponding vessel is selected in the Scale Up Assist screen (**Figure 7**).

Sampling and analytics

We took two samples from each bioreactor daily, one in the morning and one in the evening, to check offline values such as cell density, viability, glucose, ammonia (NH_3), lactate, and hmAb concentration. To collect the highest-quality sample from the growing culture in our BioBLU bioreactors, we connected a sterile 5 mL syringe to the sample port Luer Lock and removed a dead volume of 3 mL. We then collected a second 3 mL sample in a new syringe to provide a fresh, viable sample for analytics [3]. For our 40 L SUB run, we used sterile 50 mL Labtainer™ BioProcess Container bags as our sampling bags and welded them onto our sampling line using our Terumo tube welder. We first welded on a fresh bag, used to remove a dead volume of 20-30 mL from our sampling line. We then welded this bag off and welded on a new bag to our sampling line. We took roughly the same amount of sample for our cell count and analytics. Lastly, we welded on a final bag so that we could weld off the bag needed for our sampling. This is designated as the next sample time's dead volume bag. We measured cell density and viability (via the trypan blue exclusion method) using a Vi-Cell® XR Viability Analyzer (Beckman-Coulter®). We confirmed our pH values offline using an Orion Star™ 8211 pH-meter (Thermo Fisher Scientific). Using the offline pH value, we restandardized the pH calibration on the controller daily, to prevent any discrepancy between online and offline measurements. We measured glucose, ammonia, glutamate, lactate, and hMAb using a Cedex® Bio Analyzer (Roche Diagnostics®) [3].

Results

Throughout our experiments, we monitored metabolic profiles twice a day to observe how our culture was growing and to intervene if we detected any changes in growth or the health of our cells. The metabolic profile for our 40 L SUB run is shown in **Figure 8** as an example. The actively growing cultures depleted the initially supplied glucose by day 5. Lactate remained under 2.5 g/L for the duration of the run. Ammonia concentrations rose every day to a toxic level of 10.2 mmol/L by day 6. We reached 164 mg/L of IgG by day 6 (**Figure 8**).

Cell growth comparisons

The cell growth curves for all three vessel sizes are shown in **Figure 9**. By using the parameters calculated by the BioFlo 720 Scale Up Assist feature, we were able to match the growth profiles across all platforms in multiple batch runs. These results demonstrate that the new Scale Up Assist feature can decrease process development time while maintaining cell yields across vessel sizes and bioreactor

Figure 8: 40 L run metabolic profile.

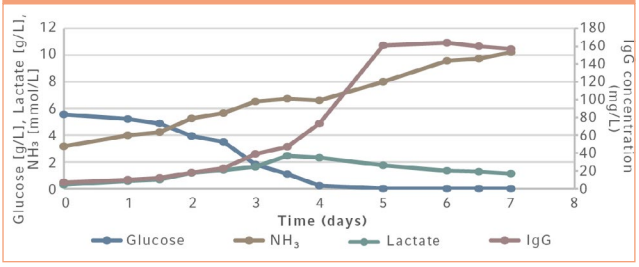
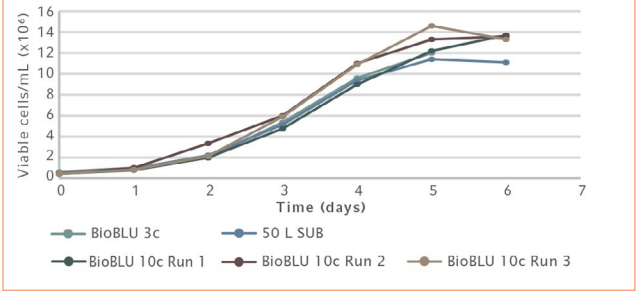


Figure 9: Cell culture comparison of BioBLU's to 50 L SUB runs.

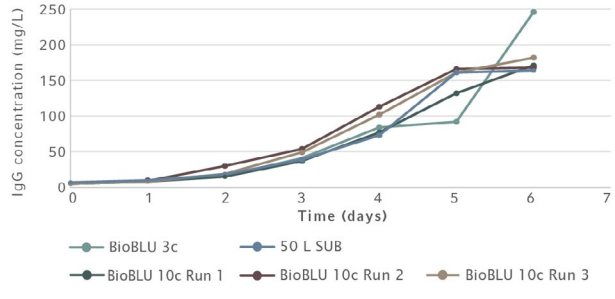


platforms. Antibody production is shown for all runs in **Figure 10**. All our runs had similar results in IgG production when they reached completion.

Conclusion

There are several possible strategies for scaling-up bioreactor cultures. Matching

Figure 10: Antibody production for scale-up runs.



K_La coefficients is one method that has been utilized with great success. This method requires extensive measurements of oxygen transfer under several different conditions. Gas flow, agitation, temperature, and bioreactor configuration can all impact K_La constants and must be taken into account. Matching impeller tip speeds is an option that simplifies the scale-up process. However, this strategy may result in reduced mixing performance in larger vessels [9]. Maintaining constant power is another straightforward scale-up strategy. All that is required is a measurement of vessel power number to calculate Equation 1. Eppendorf and Thermo Scientific have measured the power numbers for a variety of their vessels, simplifying the use of the new Eppendorf Scale Up Assist feature to scale from their Eppendorf bench-scale single-use

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vessels to Thermo Scientific's pilot and production scale single-use vessels. The BioFlo 720 Scale Up Assist application is convenient and straight forward to use. The Scale Up Assist feature allows operators to tailor their processes using constant power or tip speed. This allows users to pursue either strategy or compare them to determine which one works best for their application. Users then select their vessels from the drop-down menus. Vessel characteristics, such as impeller diameter and power numbers, are automatically programmed. These steps are facilitated through a user-defined vessel option that can be manually adapted. The Scale Up Assist then automatically calculates the critical parameters, such as agitation and maximum gassing, to successfully scale their culture from one vessel to another. In conclusion, we believe that these results demonstrate the effectiveness of the Constant Power strategy. Results that are shown using this strategy, indicate that it is possible to match yields from a 3 L to a 40 L bioreactor culture.

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