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

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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 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 1.

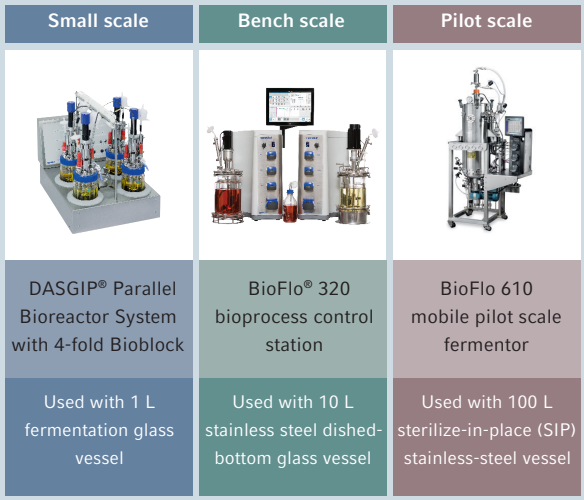

Fig. 1: Eppendorf fermentation systems, from small to pilot scale, used in this study.

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

Table 2: 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

Table 1: 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

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 2). 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]:

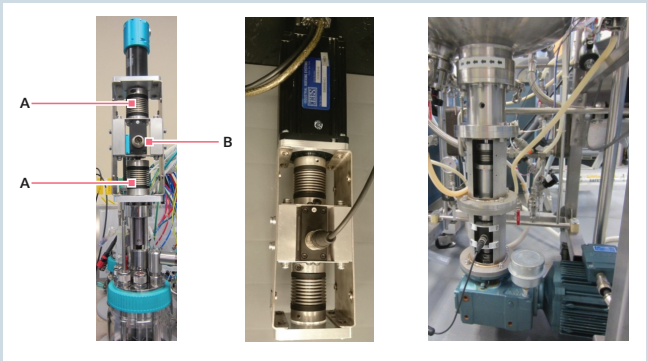

Fig. 2:
Torque sensor and adaptor setups used in DASGIP bioreactor (left), BioFlo 320 (middle), and BioFlo 610 (right).
A: The torque sensor adaptor (two pieces)
B: Torque sensor

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

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 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 $\cdot 2H_2O$, 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 3 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. 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.

Table 3: 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

Results and Discussion

As shown in Table 1, 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 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

Table 4: 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

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 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/m³, which we selected to be the constant P/V

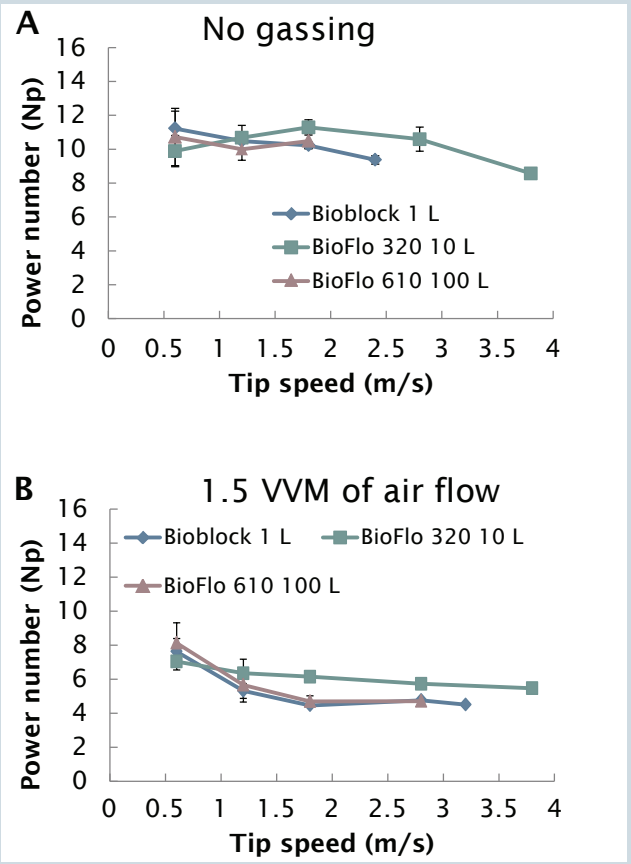

Fig. 3: Power numbers converted from impeller torque measured from DASGIP Bioblock 1 L, BioFlo 320 10 L, and BioFlo 610 100 L vessels under "no gassing" **(A)** and "high gassing" **(B)** condition. The average was plotted based on triplicate measurements. The power numbers were obtained with factory 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 5: 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

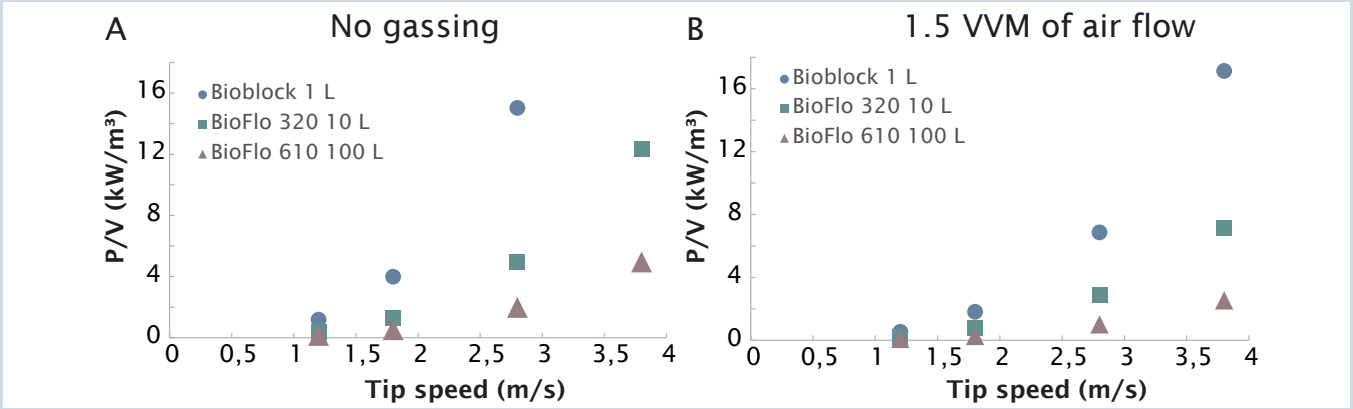

Fig. 4: P/V values calculated based on Np values from Table 5.

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. 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.

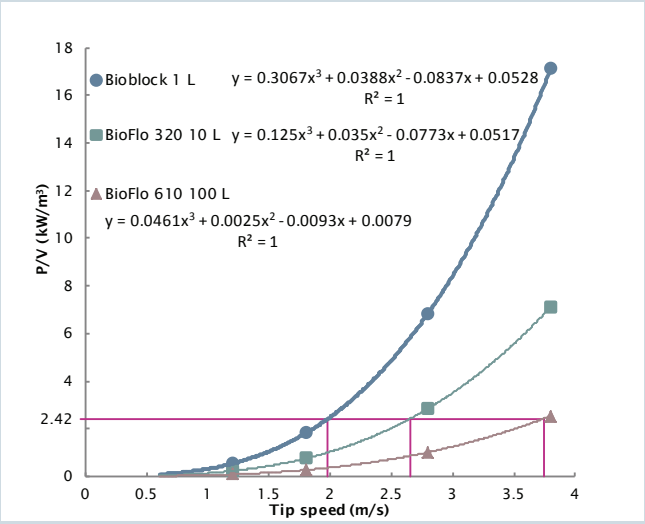

Fig. 5: Determining the constant P/V values for scale-up under 1.5 VVM of air flow

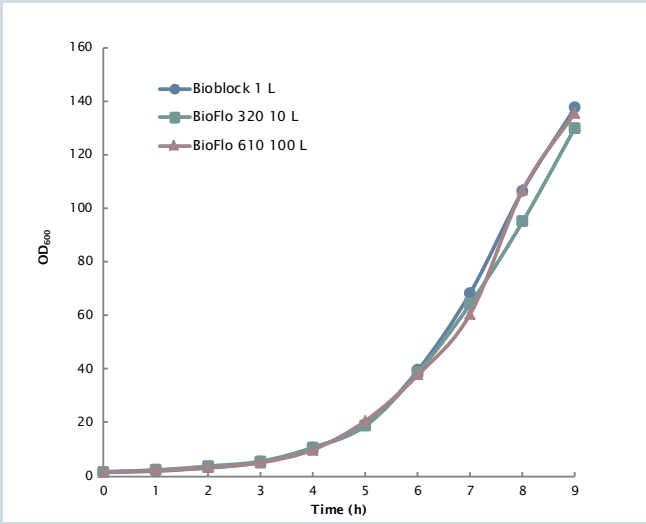

Fig. 6: Fermentation biomass growth curves among all three systems. Fermentations were carried out using a constant P/V-value of 2.42 kW/m³, which was determined from Fig. 5.

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 10L 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.

Literature

[1] How to Measure and Calculated OTR Using a New BrunswickTM Fermentor. 2013, *Applications Training Document*, Eppendorf. Inc.
[2] Löffelholz C., Husemann U., Greller G., Meusel W., Kauling J., Ay P., Kraume M., Eibl R., Eibl D. *Bioengineering* Parameters for Single-Use Bioreactors: Overview and Evaluation of Suitable Methods. *Chemie Ingenieur Technik*, 2013, 85(1-2):40-56.
[3] Geerlof A. High cell-density fermentation of *Escherichia coli*. http://www.helmholtz-muenchen.de 2008.
[4] Korz D.J., Rinas U., Hellmuth K., Sanders E.A., Deckwer W.D. Simple fed-batch technique for high cell density cultivation of *Escherichia coli*. *Journal of Biotechnology* 1995; 39(1):59-65.
[5] Raju K.S. Fluid Mechanics, Heat Transfer, and Mass Transfer: *Chemical Engineering Practice*. 2011.
[6] Zlokarnik M. S*cale-up in Chemical Engineering*. Second, Completely Revised and Extended Edition. p. 114, 2006.

Ordering information

Description	Order no.
DASGIP® Parallel Bioreactor System for Microbial Applications, max. 250 sL/h gassing	
4-fold system with Bioblock	76DG04MBBB
8-fold system with Bioblock	76DG08MBBB
16-fold system with Bioblock	76DG16MBBB
DASGIP® Bioblock Stirrer Vessels	
2x Rushton-type impeller, L-Sparger, 200 mL – 1.0 L	76SR0700ODLS
2x Rushton-type impeller, L-Sparger, 400 mL – 1.5 L	76SR1000ODLS
3x Rushton-type impeller, L-Sparger, 400 mL – 1.8 L	76SR1500ODLS
BioFlo® 320	
Base control station: All configured units include the same base control station.	1379963011
BioFlo® 320 Vessel Bundels	
1 L Vessel bundle, Stainless Steel Dish Bottom, Direct Drive	M1379-0300
3 L Vessel bundle, Stainless Steel Dish Bottom, Direct Drive	M1379-0301
5 L Vessel bundle, Stainless Steel Dish Bottom, Direct Drive	M1379-0302
10 L Vessel bundle, Stainless Steel Dish Bottom, Direct Drive	M1379-0303
1 L Vessel bundle, Water-Jacketed, Magnetic Drive	M1379-0310
3 L Vessel bundle, Water-Jacketed, Magnetic Drive	M1379-0311
5 L Vessel bundle, Water-Jacketed, Magnetic Drive	M1379-0312
10 L Vessel bundle, Water-Jacketed, Magnetic Drive	M1379-0313
New Brunswick™ BioFlo® 610	Contact us for details
New Brunswick™ Innova® 44 Shaker, 230V/50 Hz, orbit diameter 2.5 cm (1 in)	M1282-0002
Innova® U725, Ultra Low Temperature Freezer, 725 L, 230 V/50 Hz	U9449-0001
Eppendorf BioSpectrometer® kinetic, 230 V/50 – 60 Hz	6136 000.002
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