Cell Culture Scale-Up in BioBLU® c Rigid-Wall, Single-Use Bioreactors

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Abstract

For cultivation of mammalian cells in biopharmaceutical research and manufacturing, single-use technology possesses several advantages to autoclavable material. Bioreactor scalability is critical to streamlining the adaptation of culture volumes during process development and manufacturing. We analyzed BioBLU Single-Use Vessels of different sizes (maximum working volumes of 0.25 L, 3.75 L, and 40 L) that are of geometrically similar stirred-tank design. We identified a scalable tip speed zone and an overlapping range of k_La values, which cover most mammalian cell culture needs.
Using computational fluid dynamics simulations we determined the power numbers of the BioBLU bioreactors. Based on these data we scaled up a process for production of monoclonal antibodies (mAb) in CHO cells from 0.25 L to 3.75 L to 40 L by keeping constant P/V values (impeller power consumption per liquid volume) among the differently sized vessels. Similar cell growth curves and mAb production profiles were achieved at all three scales. In summary, this study demonstrates the excellent scalability of the single-use bioreactors tested.

Scope

The primary scope of this project was to investigate the scale-up capabilities of Eppendorf BioBLU Single-Use Vessels for cell culture applications from small to pilot scale.

Three bioreactor sizes were selected to represent approximately 10-fold scale-up between steps:

> **Small scale (max. 0.25 L):** 4-fold DASbox® Mini Bioreactor System with BioBLU 0.3c Single-Use Vessels.
> **Bench scale (max. 3.75 L):** BioFlo® 320 bioprocess control station with BioBLU 3c Single-Use Vessels.
> **Pilot scale (max. 40 L):** BioFlo 320 bioprocess control station with BioBLU 50c Single-Use Vessels.

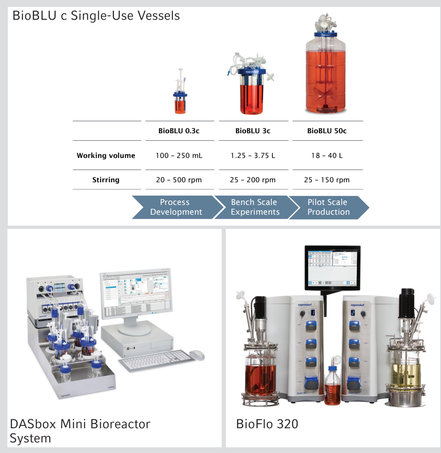

Figure 1. The equipment used in this study.

Vessel specifications

Table 1. BioBLU c Single-Use Vessel specifications.

	BioBLU 0.3c	BioBLU 3c	BioBLU 50c
Gas flow range SLPM (SLPH)	0.0007 – 0.08 (0.04 – 5)	0.002 – 1 (0.12 – 60)	0.04 – 7.5 (2.4 – 450)
Maximum gas flow (VVM)	0.33	0.27	0.19
Total volume (L)	0.38	5	50
Working volume (L)	0.1 – 0.25	1.25 – 3.75	18 – 40
Working volume : total volume	0.66	0.75	0.80
V_{max} height* (mm)	82.0	220.7	428.0
Vessel inner diameter (ID) (mm)	67.0	147.1	337.0
Vmax height : vessel ID	1.2	1.5	1.3
Vessel height : vessel ID	1.8	2.0	2.0

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

Table 2. BioBLU c Single-Use Vessels. Impeller specifications.

	BioBLU 0.3c	BioBLU 3c	BioBLU 50c
Impeller style	Pitched-blade	Pitched-blade	Pitched-blade
Impeller material	Polycarbonate	Polycarbonate	Polycarbonate
Impeller quantity	1	1	1
Impeller diameter (mm)	33	66	160
Impeller diameter : Vessel ID	0.5	0.5	0.5
Impeller height (mm)	25	50	120
Agitation (rpm)	20 – 500	25 – 200	25 – 150
Maximum tip speed (m/s)	0.9	0.7	1.3

Table 3. Tip speed vs. agitation.

Tip speed (m/s)	Agitation (rpm)		
	BioBLU 0.3c	BioBLU 3c	BioBLU 50c
0.1	58	29	12*
0.2	116	58	24*
0.3	174	87	36
0.4	231	115	48
0.5	290	145	60
0.6	347	174	72
0.7	405	200	84
0.8	465	231*	96
0.9	520	260*	108

Scalable tip speed zone: 0.3 – 0.7 m/s

* Agitation at this tip speed can not be achieved by this vessel (beyond vessel spec.)

The Eppendorf BioBLU single-use cell culture vessels from small scale to pilot scale are of geometrically and proportionally similar stirred-tank designs.
A wide scalable tip speed zone (0.3 – 0.7 m/s) has been identified which covers most mammalian cell culture needs.

Oxygen transfer

Table 4. Measurement conditions for the determination of k_La values.

	BioBLU 0.3c (Open pipe sparger)					BioBLU 3c (Macrosparger)					BioBLU 50c (Macrosparger)				
	Working volume 0.25 L					Working volume 3.75 L					Working volume 40 L				
Tip speed (m/s)	0.3	0.4	0.5	0.6	0.7	0.3	0.4	0.5	0.6	0.7	0.3	0.4	0.5	0.6	0.7
Agitation (rpm)	174	231	290	347	405	87	115	145	174	203	36	48	60	72	80
VVM	Air flow (SLPM)					Air flow (SLPM)					Air flow (SLPM)				
0.01	0.0025					0.0375					0.4				
0.03	0.0075					0.1125					1.2				
0.05	0.0125					0.1875					2.0				
0.10	0.0250					0.375					4.0				
0.15	0.0375					0.5625					6.0				

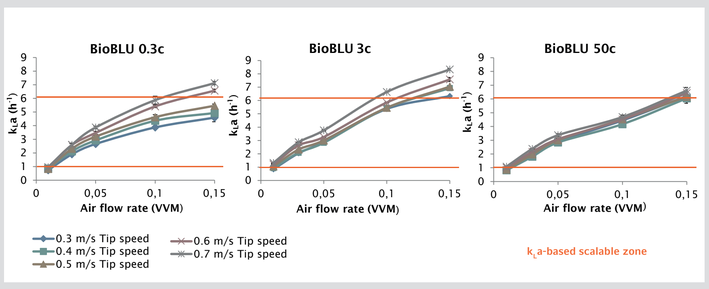

Figure 2. BioBLU Single-Use Vessel k_La values.

Maintaining a constant k_La between vessels of different sizes is one of the frequently used strategies for cell culture scale-up. It is important to select equipment with similar k_La capabilities that offer sufficient overlapping so that the small scale success can be replicated in large scale. k_La-based scale-up can be performed by maintaining constant k_La values among different vessels in the 0.8 – 6.0 h^{-1} range, providing flexibility to accommodate different types of cell lines.

Constant P/V scale-up

Using computational fluid dynamics (CFD) simulations the dimensionless power number Np was determined for different BioBLU vessels at different stirrer speeds.

Table 5. Power numbers obtained by CFD from 0.3 – 0.7 tip speeds (one impeller, up flow, no gassing).

Bioreactor	Tip speed (m/s)	Np	Mean Np
BioBLU 0.3	0.3	2.64	2.54
	0.4	2.57	
	0.5	2.52	
	0.6	2.49	
	0.7	2.46	
BioBLU 3c	0.3	2.62	2.56
	0.4	2.58	
	0.5	2.55	
	0.6	2.53	
	0.7	2.52	
BioBLU 50c	0.3	2.52	2.52
	0.4	2.52	
	0.5	2.52	
	0.6	2.53	
	0.7	2.53	

The purpose of determining Np is to calculate impeller power consumption per liquid volume (P/V, W/m^3). Maintaining constant P/V between vessels is one of the most accepted strategies for scale-up. P/V can be converted from power number (Np) using the following equation:

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

ρ: DI water density, 1,000 kg/m^3
N: agitation speed, rps
d: impeller OD, m
V: full working volume, m^3

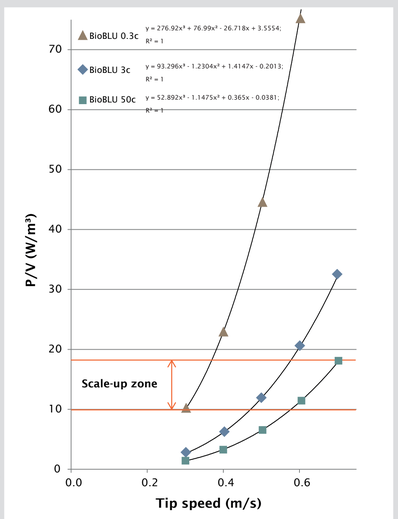

Figure 3. Among the different vessels P/V can be kept constant in the range of 10.0 – 18.2 W/m^3.

CHO culture and mAb production was scaled up with a constant P/V of 10.9 W/m^3. This lower P/V value within the scale-up zone was chosen to reduce tip speed/shearing. Scalable growth profiles and similar mAb production profiles were achieved from 0.25 L to 40 L.

Table 6. CHO cell culture. Tip speed and agitation at the different scales.

Bioreactor	BioBLU 0.3c	BioBLU 3c	BioBLU 50c
Tip speed (m/s)	0.30	0.47	0.58
Agitation (rpm)	174	137	69

Table 7. CHO cell culture. Experimental conditions for all scales.

CHO culture	Conditions for all scales
P/V	10.9 W/m^3
Air flow mode	3-Gas Auto
Maximum gassing	0.19 VVM
pH/deadband	7.0/0.1
DO	50 %
Temperature	37°C
Working volume	Maximum working volume (0.25 L I 3.75 L I 40 L)

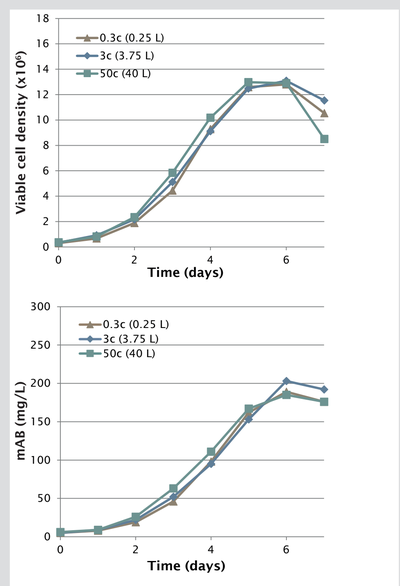

Figure 4. Scale-up of a mAb production process with CHO cells. Viable cell density (top) and antibody concentration (bottom) were analyzed at the different scales.

Conclusion

> The Eppendorf BioBLU c Single-Use Vessels are of geometrically and proportionally similar stirred-tank designs.
> A wide scalable tip speed zone has been identified which covers most mammalian cell culture needs.
> All three vessel sizes offer a broad overlapping range of k_La values for excellent scalability.
> P/V-based scale-up can be performed by maintaining constant P/V values among different vessels from 10.0 to 18.2 W/m^3.
> A CHO cell culture process was scaled-up from 0.25 L to 40 L using the constant P/V strategy. Similar cell growth curves and mAb production yield profiles were achieved at the different scales.

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