Constant P/V-Based Scale-Up of Packed-Bed Perfusion Bioreactors for Culture of Adherent Cells

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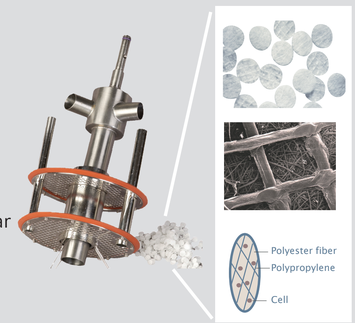
Abstract

Fibra-Cel® packed-bed bioreactors have been used successfully in high-yield vaccine and gene therapy perfusion processes for many years. While much has been done on the characterization of scale-up for stirred-tank bioreactors, relatively little is known on the scalability of packed-bed bioreactors for culture of adherent cells. Here we describe our approach to accurately determine packed-bed vessel power numbers of bench-scale single-use bioreactors and vaccine production-scale sterilize-in-place (SIP) bioreactors to enable constant power per volume (P/V) scale-up. Cell cultures under constant P/V among different scales have been shown to reliably produce comparable yields. We intend to show the technique used to remove mechanical resistance from the agitation shaft in order to obtain accurate rotational torque measurement for the determination of power numbers. We also investigate the relationship between a single-use 5 L packed-bed bioreactor (BioBLU® 5p Single-Use Vessel with BioFlo® 320 bioprocess controller), and a stainless steel 32 L vessel (CelliGen® 510). The power numbers are measured on both systems and used to convert into P/V values at different tip speeds. An analysis is provided to enable constant P/V scale-up using packed-bed vessels equipped with a Fibra-Cel basket impeller.

Packed-bed bioreactors

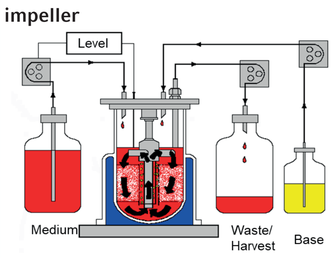
Basket impeller and Fibra-Cel disks

Fibra-Cel:
> Disks of 6 mm diameter, made of polyester non-woven fiber and polypropylene
> For attachment or suspension cells
> Provides a very low-shear environment
> High surface to volume ratio
> Materials of construction are USP Class VI-certified

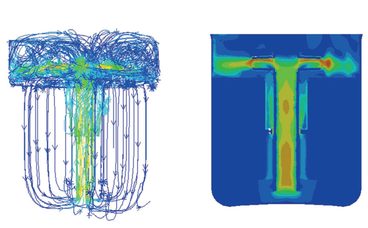


Packed-bed bioreactors

Perfusion with the Fibra-Cel basket impeller



Agitation and Flow modeling by CFD



The impeller rotation creates centrifugal force driving medium to circulate uniformly through the entire basket.

Advantages
> Reduced shear force
> High mass transfer of nutrients
> For use with suspension cells as well as anchorage-dependent cells
> Medium exchange without cell loss

Scale-up capabilities

BioFlo 320 with BioBLU 5p Singe-Use Vessel

CelliGen 510 SIP unit with built-in packed-bed system

~9-fold scale-up

Vessel characteristics

	BioBLU 5p Single-Use Vessel	CelliGen 510 packed-bed (SIP)
Gas flow range (SLPM)	0 – 7.5	0 – 64
Max. gas flow (VVM)	2	2
Total volume (L)	5	40
Working volume (L)	3.75	32
Working volume : total volume	0.75	0.8
V_{max} height* (mm)	192	451
Vessel inner diameter (ID) (mm)	166	302
V_{max} height : vessel ID	1.2	1.5
Vessel height : vessel ID	1.5	2.0
Fibra-Cel	150 g	1,200 g

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

Impeller characteristics

	BioBLU 5p Single-Use Vessel	CelliGen 510 packed-bed (SIP)
Style	Cell-lift basket impeller	Cell-lift basket impeller
Material	Polycarbonate	Stainless steel
Quantity	1	1
Diameter (mm)	80.25	177.8
Impeller diameter : vessel ID	0.48	0.59
Agitation (rpm)	20 – 200	25 – 200
Max. tip speed (m/s)	0.8	2.4

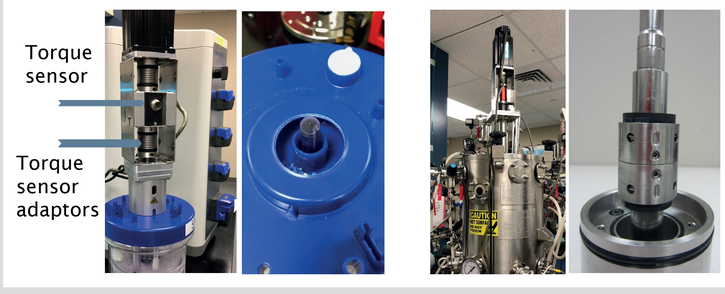
Tip speed vs. agitation correlation

Tip speed (m/s)	BioBLU 5p (rpm)	CelliGen 510 packed-bed (rpm)
0.2	48	21*
0.3	71	32
0.4	95	43
0.5	119	54
0.6	143	64
0.7	167	75
0.8	190	86
0.9	214*	97
1.0	238*	107
1.1	262*	118

Constant tip speed scale-up zone

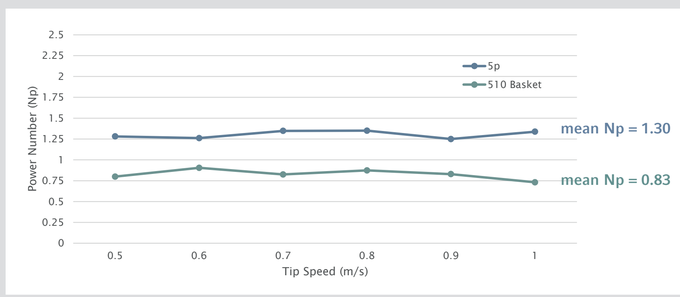
* Agitation at this tip speed cannot be achieved by this vessel (beyond vessel specification)

Power number and scale-up



A torque sensor is connected in between the motor and impeller shaft using custom adaptors and bearing modifications. Left: BioBLU 5p Single-Use Vessel. Right: CelliGen 510.

Torque data were collected according to DECHMA recommendations [2]
> All data, including dead weight torque, were collected using a sample rate of 5 samples per second for 60 seconds, giving a total of 300 readings per measurement (DECHMA recommends at least 60 readings per measurement).
> Impeller working torque measurements were repeated five times at each tip speed with no aeration (DECHMA recommends at least 3 measurements without aeration for cell culture applications).
> DECHMA states that for cell culture conditions, measurements with aeration are not necessary as gassing rates are typically very low.
> Data collection was done at ambient temperature of 24.5 °C (DECHMA recommends 25 °C ± 0.5 °C)

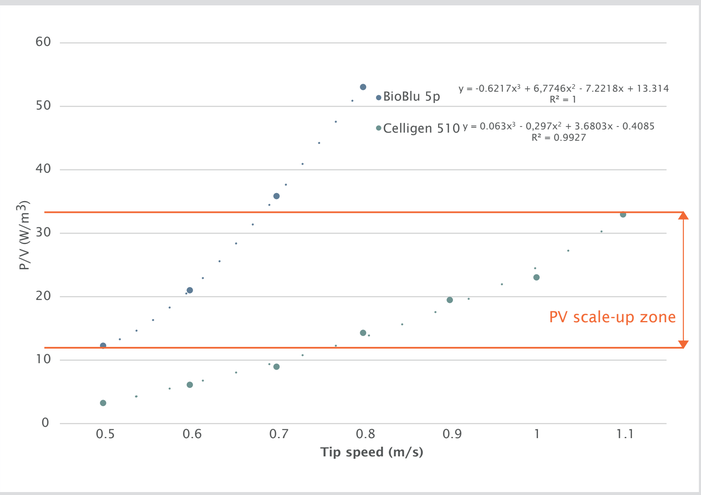


Experimentally derived power numbers for BioBLU 5p and CelliGen 510 packed-bed vessels equipped with cell-lift basket impeller fully loaded with Fibra-Cel.

P/V can be converted from power number (Np):

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

ρ: DI water density, 1000 kg/m³
N: agitation speed, rps
d: impeller OD, m
V: full working volume, m³



P/V scale-up zone

Tip speed
> Commonly used parameter for scale-up, maintaining a relatively constant shear force level
> Constant tip speed may result in different impeller power input for differently sized vessels; adjustments may be necessary to manage a scalable yield
> Tip speed scalable zone is used to guide the agitation range for power number determination [1]

Power number
> Also known as Newton number, Ne; dimensionless number associated with each type of impeller
> Commonly used to calculate impeller and vessel power consumption during bioprocess scale-up; the industry standard-way to determine Np is to measure impeller torque by using a rotational torque sensor
> Important to measure net impeller torque without any bearing resistance; impeller torque = torque value measured in water at given agitation speed minus the torque value of empty vessel (dead weight torque) at the same agitation speed
> To reduce bearing resistance, the magnetic drive coupling in BioBLU 5p was removed and converted to direct-drive. The double mechanical seals in CelliGen 510 bearing assembly were also removed
> In both cases, the torque measurement device was directly connected to the impeller shaft

Conclusion

> The Eppendorf BioBLU 5p Single-Use Vessel and the CelliGen 510 packed-bed vessels are designed with similar geometries.
> The two vessels have a wide range of overlapping tip speeds to allow constant tip speed-based scale-up to be conducted.
> The two vessels have a wide range of overlapping P/V values under typical cell culture conditions. Constant P/V-based scale-up between packed-bed vessels can be performed in the range from 12 to 33 W/m³.
> Additional studies are underway to conduct perfusion culture of Vero cells in BioBLU 5p and CelliGen 510 following constant P/V strategy.

References

[1] Bin L., Sha M., Scale-up of Escherichia coli Fermentation From Small Scale to Pilot Scale Using Eppendorf Fermentation Systems. Eppendorf Application Note #306.
[2] Recommendations for process engineering characterization of single-use bioreactors and mixing systems by using experimental methods. DECHMA 2016