

Scale-Up of a Biosimilar Production Process with CHO Cells from Small to Bench Scale

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Abstract

Bioprocess scale-up from small development scale to pilot and production scale is a fundamental part of process development in the biopharmaceutical industry. To reproduce process performance while increasing working volume, the optimal cellular environment must be replicated.

Scale-up often comes along with transference of the process to bioprocess systems with other properties, such as different geometries and engineering parameters. Depending on the scale-up strategy, parameters such as mixing time, oxygen transfer, and power input are to be considered. As it is usually not feasible to conserve all the parameters when scaling up, bioengineers must assess which are the most critical. Among the most important

aspects of scale-up are oxygen mass transfer and the dissolved oxygen (DO) concentration in the cultivation medium. Oxygen mass transfer depends mainly on the air sparge system, the gassing rate, oxygen concentration, selected impellers, their settings, power input and properties for gas dispersion.

In this study, we scaled up a CHO cell culture bioprocess from 1 L using a DASGIP® Parallel Bioreactor System to 5 L using a BioFlo® 320 Bioprocess Control Station. To develop a scale-up strategy, we partly compared power number and volumetric mass transfer coefficient ($k_L a$) of the bioreactors. The results show that $k_L a$ value can be used as a suitable scale-up criterion from small to bench-scale.

Introduction

Monoclonal antibodies and therapeutic proteins are an important segment for the biotech industry. The execution of a successful bioprocess scale-up and transfer of technology and activities enables a fast time to market approach, supply chain risk mitigations, and market access [1]. Also, from a regulatory perspective, the Quality by Design (QbD) approach that is promoted by the EMA and FDA requires detailed process and product understanding gained by a successful scale-up as well as a clear definition of critical quality attributes and critical process parameters.

In the development of biosimilars, small bioreactors save time, material, and resources. With the ability to work in parallel, the DASGIP Parallel Bioreactor System equipped

with 1 L vessels can be used to optimize bioprocess parameters, including temperature, shear stress, and pH regulation. In the case of large scale production, it is important to show that the results of the parallel system can be transferred to larger scales, such as the Eppendorf 5 L vessel of the BioFlo 320 system. Another advantage is that the higher harvest volume of the larger system provides enough material to optimize the purification and the characterization of the biosimilar.

UGA Biopharma used the Chinese hamster ovary (CHO) DG44 cell line for the contract development of a Pertuzumab-expressing biosimilar cell line. To demonstrate scale up of a cell culture bioprocess from a working volume of 1 L to 5 L,

a DASGIP Parallel Bioreactor System equipped with glass vessels with a maximum working volume of 1.0 L (DASGIP 1 L vessel) and a BioFlo 320 bioprocess control station equipped with a glass vessel with a maximum working volume of 5.6 L (BioFlo 320 5 L vessel) were used.

In the design of an optimal scale-up process, several parameters are important: Design of the bioreactor, impeller diameter, tip speed, volumetric mass transfer coefficient ($k_L a$), mixing time, and power number. The bioreactors employed in the scale up process differ in geometry and functional assemblies, e.g. impellers and sparger type. In the 1 L vessel a L-sparger was used instead of the standard open pipe dip tube. For a better comparison, a L-sparger was tested in the 5 L vessel in addition to the standard ring sparger.

The $k_L a$ – the volumetric oxygen mass transfer coefficient

– is used as a key performance parameter in biotech engineering. The $k_L a$ is defined as the reciprocal time for the transfer of oxygen from the gaseous to the liquid phase [2]. Here, k_L defines the liquid side mass transfer coefficient, in which the resistance in the gas side film is assumed to be neglectable and a defines the bubble surface that is physically available for oxygen diffusion.

More Information

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Cell culture bioprocess scale-up workflow from bench to pilot scale

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Material and Methods

Cell line, medium, inoculation and basic fed-batch settings

CHO DG44 cells producing a Pertuzumab biosimilar molecule were developed and provided by UGA Biopharma. The cell banks were stored at $-80\text{ }^{\circ}\text{C}$. For usage, the cells were thawed, cultivated in suspension and the number of cells were expanded in shake flasks from 25 mL up to 220 mL at $37\text{ }^{\circ}\text{C}$, 150 rpm, 50 mm orbit, and 8 % CO_2 . Afterwards, all stirred-tank bioreactors were seeded with 4.5×10^5 cells/mL. Commercially available, fully chemically defined, animal component-free First CHOice® Medium and Feeds were used for the cultivation of the Pertuzumab expressing biosimilar cell line in shake flasks and bioreactors. The daily feed was started at day 3. The glucose level were controlled every day and an additional glucose stock solution was feeded on demand. The fed-batch was finalized after a viability drop down to $\leq 75\text{ }%$.

Bioprocess system and process parameters

For the cultivation in stirred tank bioreactors, two Eppendorf bioreactor control systems were used. First, the DASGIP Parallel Bioreactor System with Bioblock (Figure 1A) was used as a parallel modular system with 1 L glass vessels using as a modified setup an additional Rushton-type impeller. Secondly, a BioFlo 320 bioprocess control station (Figure 1B) with 5 L glass vessel also with a modified vessel setup was employed.

The pH was regulated by the addition of sodium bicarbonate and CO_2 . The DO was controlled by an automatic



Fig. 1A: DASGIP Parallel Bioreactor System with Bioblock



Fig. 1B: BioFlo 320 bioprocess control station

Table 1: Vessel configuration and information

	DASGIP vessel, 1 L (DS1000DSS)		BioFlo 320 vessel, 5 L (M1379-1007)	
	Standard	Used modification	Standard	Used modification
Drive connection	Direct drive	Direct drive	Direct drive	Direct drive
Tempering	Heating well	Heating well	Water jacket	Water jacket
Total vessel volume [L]	1.8	1.8	7.5	7.5
Maximum working volume [L]	1.0	1.0	5.6	5.6
Vessel height (H) [mm]	241	241	326	326
Vessel diameter (D) [mm]	100	100	176	176
H/D	2.4	2.4	1.85	1.85
Type of bottom stirrer	Pitched blade	Pitched blade	Pitched blade	Pitched blade
Amount of blades	3	3	3	3
Impeller blade angle [°]	30	30	45	45
Impeller diameter (d) [mm]	50.5	50.5	88.9	88.9
Impeller height (h) [mm]	27.5	27.5	62.8	62.8
Impeller setup height (h0) [mm]	16.7	25	88.9	81
h0/D	0.167	0.25		
Type of upper stirrer	Pitched blade	Rushton type	—	Rushton type
Amount of blades	3	6	—	6
Impeller blade angle [mm]	30	90	—	90
Impeller diameter (d) [mm]	50.5	46	—	70.4
Impeller height (h) [mm]	27.5	12	—	17.6
Impeller setup height (h1) [mm]	77.2	75	—	141
h1/D	0.772	0.75	—	0.80
Dip tube for sampling	Li 220 mm	Li 220 mm	Li 305 mm	Li 305 mm
Dip tube for harvest	Li 222 mm	Li 222 mm	Li 320 mm	Li 320 mm
Sparger	Open pipe	L-sparger	Ring sparger	L-sparger
pH-sensor	Li 225 mm	Li 225 mm	Li 325 mm	Li 325 mm
DO-sensor	Li 225 mm	Li 225 mm	Li 325 mm	Li 325 mm

gas mix of air, oxygen and nitrogen. The gas mix module for the parallel bioreactor system MX4/4 consists of four thermal mass flow controllers (TMFCs) and four valves. The BioFlo320's gas mix module is built from one TMFC and four valves.

Vessel configurations are summarized in Table 1 and Table 2.

Determination of the power-number

The determination of the power-number (N_p), also known as Newton-number (Ne), was performed based on the torque measurement as it is described in the DECHEMA® instructions for characterization of single-use equipment [1]. The torque sensor was mounted between the shaft and the drive unit.

$$P = N_p \times \rho \times N^3 d^5$$

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

$$\text{Power Number } (N_p) = \frac{2\pi N(M - M_0)}{\rho N^3 d^5}$$

$$\text{Power Number } (N_p) = \frac{2\pi M_{\text{netto}}}{\rho N^2 d^5}$$

P: impeller power consumption (W)
 M: Torque (with full working volume of DI water), (N-m)
 M_0 : Torque (empty vessel), (N-m)
 M_{netto} : $M - M_0$ (N-m)
 ρ : DI water density = 1,000 kg/m³
 N: Agitation speed (rps)
 d: Impeller outer diameter (m)
 V: Full working volume (m³)

Equations: Impeller power consumption per volume and power number

Table 2: Specific vessel process configuration

Bioprocess control system	DASGIP Parallel Bioreactor System with Bioblock	BioFlo 320 Bioprocess Control Station
Vessel maximum working volume [L]	1.0 L	5.6 L
Culture method	Fed batch	Fed batch
Growth medium	First CHOice	First CHOice
Initial working volume [L]	0.55	3.5
Maximum used working volume [L]	1	5
Temperature setpoint [°C]	37	37
pH setpoint	6.9	6.9
Acid	CO ₂	CO ₂
Base	Na(HCO ₃) ₂ [1 M]	Na(HCO ₃) ₂ [1 M]
DO cascade setpoint [%]	40	40
Agitation [rpm]	120	140
Tip speed [m/s]	0.32	0.65
Gas flow [sL/h]	1	4 – 16
O₂ concentration [%]	21 – 100	21 – 100

As a torque meter, a DRFL-I (ETH-Messtechnik GmbH, Gschwend, Germany), with a torque range of 0 – 0.5 Nm, was used to derive the torque data for the 1 L vessel. To ensure an installation in the correct position and configured to provide accurate measurement, bellows couplings were used to couple the torque sensor to the motor and to the stirrer shaft side. This is necessary to eliminate forces through different misalignments which can occur in the axial, angular, and radial positions [1]. The power supply for the torque sensor was realized by an external 12 V power converter. The torque sensor was electrically connected for the signal to the analogue 0 to 10 V input/output port of the Eppendorf pump module DASGIP MP8. The Eppendorf DASware® control was used adopted for data storage. For the torque measurement of the BioFlo 320 5 L vessel, the torque sensor TRS600 from FUTEK, with a measuring range of 0-1 Nm, was used. The sensor's analogue signal was converted via the USB320 module, while data storage was carried out with FUTEK's own data acquisition software (known by the acronym SENSIT) for test and measurement. The control of the agitation speed was automated by the BioCommand® Batch Control and defined profiles and visual basic scripts. Prior to every measurement step the base line of the torque's voltage signal was measured from the ETH torque sensor while concurrently the base line torque was

measured from the FUTEK torque sensor. A minimum of 30 datapoints were recorded for later analysis. For the torque under load several agitation speeds were used. The agitation tip speed includes a range from 0.3 m/s up to 2 m/s with the 5 L vessel and 3 m/s for the 1L vessel. A minimum of 60 datapoints were measured for the torque under load during a measurement period of at least 15 minutes. The vessels were configured as they were used in the process. The stirring direction was counter clockwise stirring with the up flow in the position as defined as the standard.

To calculate the power number for the two-stage stirred system, the average diameter of the impeller was used. To calculate the power numbers the values determined in turbulent flow regime were used, starting from 0.5 to 2.5 m/s tip speed when the power-numbers have been constant. To derive the netto-torque value M_{netto} , the torque data from the drained vessel M_{drained} was subtracted from the torque of the filled vessel M .

Determination of the $k_L a$

For the determination of the $k_L a$, the gassing-out method was used. In this application, the dissolved oxygen in the fluid is measured via a standard amperometric sensor. Nitrogen was used to strip-out the oxygen dissolved within the liquid. The strip-out phase was followed by the aeration phase. The data acquisition was performed using the DASware control software. The sample rate was set to 5 seconds to obtain accurate results. A 1 % NaCl solution was used to mimic the coalescence property of the cell culture media. The bioreactors were filled to different working volumes appropriate to the fed batch process – filling to immerse the lowest impeller, filling to immerse the upper impeller, filling up to the maximum working volume. The distinct bioreactor control systems DASware control and BioCommand Track and Trend were used for data acquisition.

To establish the measurement parameters, the bioreactor was filled to the desired volume with the vessel setup as defined by the process. The dissolved amperometric oxygen sensor (Clark-sensor) was connected to the bioprocess controller BioFlo 320 or the sensor module PH4PO4 for the DASGIP Parallel Bioreactor System in order to polarize the DO sensor for at least 6 h prior to starting the measurement. The 1 % NaCl solution was brought to a constant temperature of 37 °C (±0.5 °C) and the agitating was activated. For a two-point calibration the bioreactor and NaCl solution were sparged with nitrogen and air. For more information on DO sensor calibration see the

DASware control and/or BioCommand manual. To eliminate the dissolved oxygen (zero/offset calibration) the 1 % NaCl solution was sparged with nitrogen. Once a constant DO value was achieved, the DO value was calibrated to 0 %. At this point the nitrogen supply was stopped and the air supply initiated to calibrate under maximum DO conditions to 100 % DO until a stable signal was achieved. The gassing in and gassing out steps were defined by profile tables for setting the XO2.SP to 0 % O₂ and 21 % O₂ in the inlet gas stream. It was ensured that the values near 0 % DO and

100 % DO were reached.

Analytcs

Viablc cell density and viability were measured and calculated with the Luna™ Automated Cell Counter as per the manufacturer’s recommendations. The amount of synthesized protein was measured by Protein A-HPLC. Glucose and lactate concentrations were measured using Biosen® analyzer (EKF Diagnostics, Germany).

Results

Scale-up strategy

For the system and process scale-up the power-number, tip-speed, and k_La values were evaluated. The impeller speed and the assemblies of the BioFlo 320 system were also optimized during the experiments.

Tip speed

The scale-up using the stirrers tip speed is programmed to achieve on a constant stirrer circumferential speed v throughout the scales.

$$v = d\pi n$$

Table 3: Tip speed calculatins of 1 L and 5 L vessel

Tip speed [m/s]	DASGIP vessel, 1 L	BioFlo 320 vessel, 5 L
	Agitation speed [rpm]	
0.05	19	11
0.1	38	21
0.15	57	32
0.2	76	43
0.25	95	54
0.3	113	64
0.32	121	69
0.35	132	75
0.4	151	86
0.45	170	97
0.5	189	107
0.55	208	118
0.6	227	129
0.65	246	140

The tip speed of the 1 L vessel’s pitched-blade with 120 rpm corresponds to approximately 0.32 m/s. That will result in an agitation speed of around 69 rpm for the 5 L vessel (Table 3).

Using this scale-up approach led to observably less gas bubble dispersion in the bioreactor.

Power number

The power-number of the pitched blade of the 1 L vessel is lower than the power-number of the pitched blade of the 5 L vessel (Table 4). As the process reached the maximum

Table 4: Power numbers of 1 L and 5 L vessel stirrer setups

Impeller setting	DASGIP vessel, 1 L	BioFlo 320 vessel, 5 L
1 x pitched blade impeller	0.9 ± 0.1 W	2.3 ± 0.2 W
2 x pitched blade impeller	1.9 ± 0.4 W	n.d
1 x pitched blade impeller 1 x Rushton-type	4.14 ± 0.1 W	4.3 ± 0.1 W

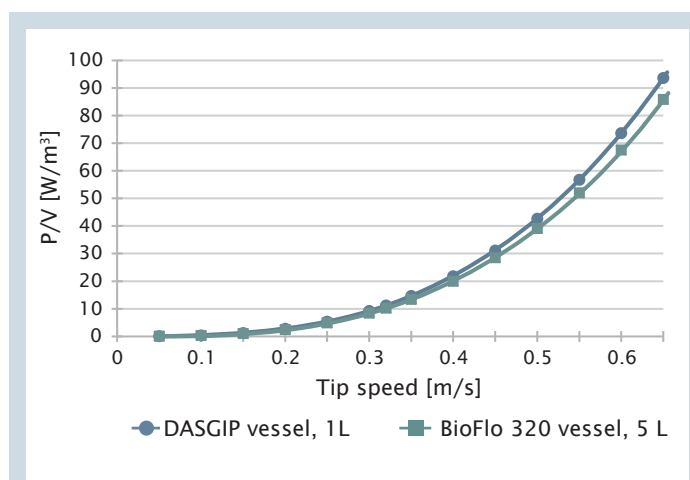


Fig. 2: Power input per volume of 1 L vs. 5 L vessel stirrer setup with pitched blade impeller and Rushton-type impeller setup.

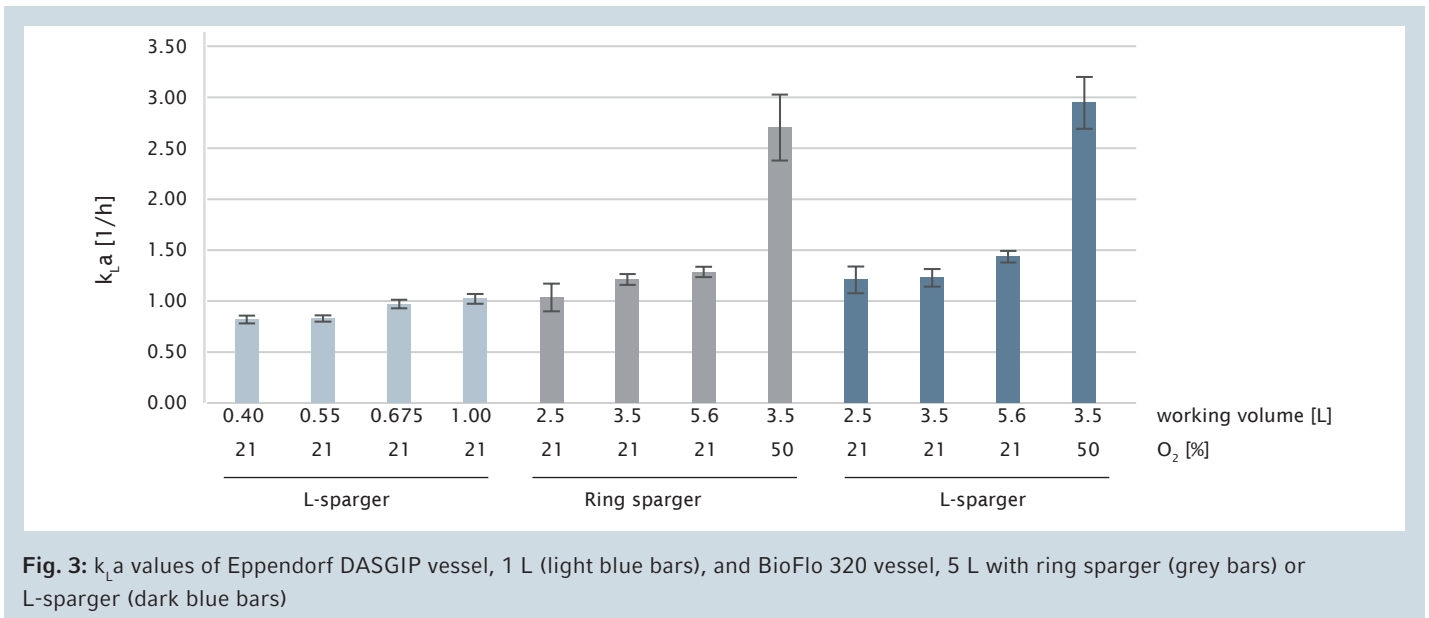


Fig. 3: $k_L a$ values of Eppendorf DASGIP vessel, 1 L (light blue bars), and BioFlo 320 vessel, 5 L with ring sparger (grey bars) or L-sparger (dark blue bars)

Table 5: $k_L a$ values for different process conditions

Vessel type	Working volume [L]	Sparger	Oxygen [%]	Gas flow [sL/h]	Agitation [rpm]	Tip speed [m/s]	Average $k_L a$ [1/h]	Standard deviation [\pm 1/h]
DASGIP vessel, 1 L	0.40	L-sparger	21	1	120	0.32	0.82	\pm 0.04
DASGIP vessel, 1 L	0.55	L-sparger	21	1	120	0.32	0.83	\pm 0.03
DASGIP vessel, 1 L	0.675	L-sparger	21	1	120	0.32	0.97	\pm 0.04
DASGIP vessel, 1 L	1.00	L-sparger	21	1	120	0.32	1.02	\pm 0.05
BioFlo 320 vessel, 5 L	2.5	Ring sparger	21	4	140	0.65	1.04	\pm 0.14
BioFlo 320 vessel, 5 L	3.5	Ring sparger	21	4	140	0.65	1.21	\pm 0.05
BioFlo 320 vessel, 5 L	5.6	Ring sparger	21	4	140	0.65	1.29	\pm 0.05
BioFlo 320 vessel, 5 L	3.5	Ring sparger	50	8	180	0.84	2.70	\pm 0.32
BioFlo 320 vessel, 5 L	2.5	L-sparger	21	4	140	0.65	1.21	\pm 0.13
BioFlo 320 vessel, 5 L	3.5	L-sparger	21	4	140	0.65	1.23	\pm 0.09
BioFlo 320 vessel, 5 L	5.6	L-sparger	21	4	140	0.65	1.44	\pm 0.06
BioFlo 320 vessel, 5 L	3.5	L-sparger	50	8	140	0.84	2.95	\pm 0.26

Remark: Tip speed calculation is based on pitched blade impeller diameter

working volume and the two impellers were immersed the power numbers became more equal. Comparing the power input at equal tip speeds the power input would be comparable (Figure 2). However, the power input for the 1 L vessel at 120 rpm, 0.55L with ca. 11 W/m^3 is lower than for the 5 L vessel at 3.5 L working volume using 140 rpm with about 86 W/m^3 .

Volumetric mass transfer coefficient

The ring sparger was identified as a bioreactor component that has a potential influence on the $k_L a$ value and a part that can be tested to be exchanged. To check if another gassing system would be beneficial, the L-sparger of the 4 L vessel (76DR04C) of the modular DASGIP Parallel Bioreactor System was used for comparison to the 5 L ring sparger setup. Note that the benchtop vessel dimensions are based on the Anglo-American measurement systems while the

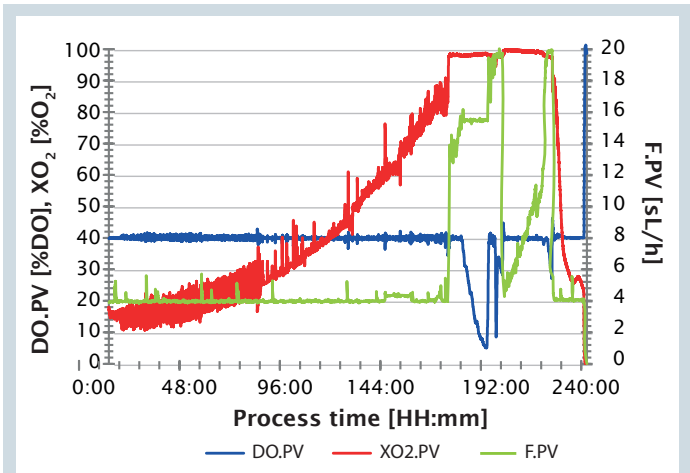


Fig. 4: Dissolved oxygen, oxygen concentration and gas flow in 5 L vessel with ring sparger at 100 rpm.
 DO.PV: Process value dissolved oxygen. XO2.PV: Process value oxygen concentration in gas mix. F.PV: Process value gas flow

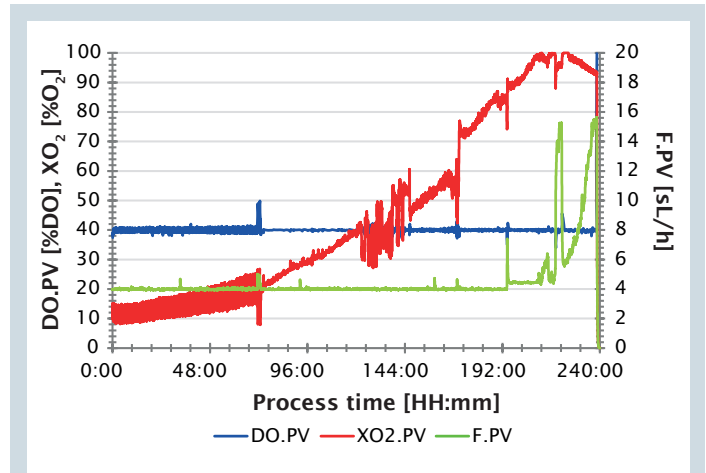


Fig. 5: Dissolved oxygen, oxygen concentration and gas flow in 5 L vessel with ring sparger at 140 rpm.
 DO.PV: Process value dissolved oxygen. XO2.PV: Process value oxygen concentration in gas mix. F.PV: Process value gas flow

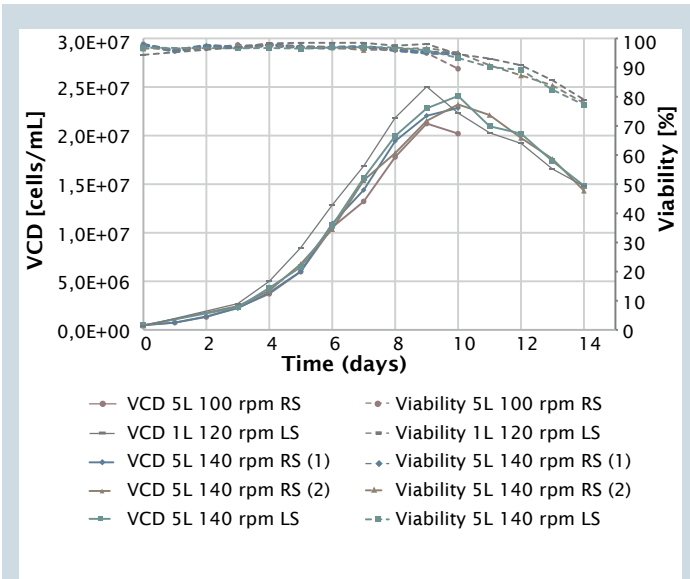


Fig. 6: Viable cell density (VCD) and viability at different process conditions in DASGIP vessel, 1L and BioFlo 320 vessel, 5 L were compared. RS: Ring sparger. LS: L-sparger. 5 L vessel, 140 rpm, RS: Results from two experimental runs are shown ((1) and (2)).

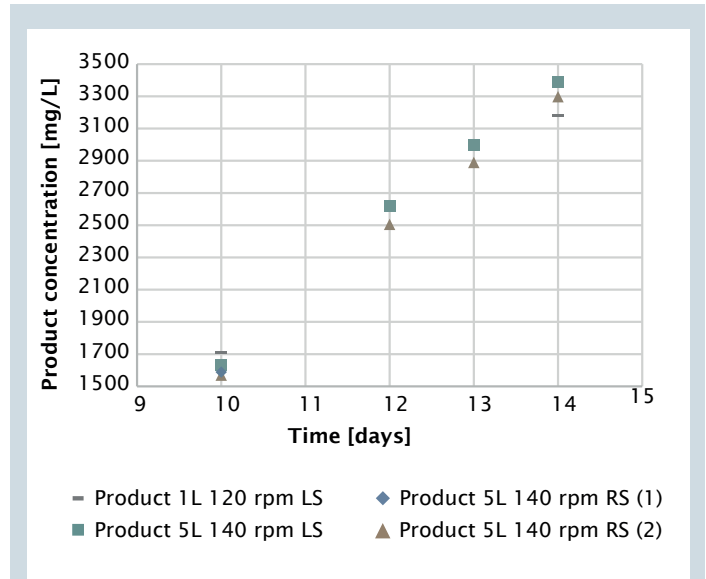


Fig. 7: Product formation at different process conditions in DASGIP vessel, 1L and BioFlo 320 vessel, 5 L were compared. RS: Ring sparger. LS: L-sparger. 5 L vessel, 140 rpm, RS: Results from two experimental runs are shown ((1) and (2)).

small-scale systems are based on the metric system. Here, the used compression fittings must be carefully handled and maintained.

The $k_L a$ -data of the measurements from the ring sparger vs. the L-sparger of the BioFlo 320 5 L vessel yielded comparable results (Figure 3, Table 5). Only a slight

tendency in favour of the L-sparger could be identified but did not yield significantly better performance. However, independently from using the ring sparger or L-sparger in the 5 L vessel it is shown that the process window for the $k_L a$ of the 1 L vessel can be scaled-up to the 5 L vessel.

Cell culture

Initially, the scale-ups of the modified 5 L vessel were run at 100 rpm (0.47 m/s; 55 W/m³) and 140 rpm (0.65 m/s; 85 W/m³) with the ring sparger. The original tip speed of the 1 L DASGIP cell culture vessel was 0.32 m/s (120 rpm, 11 W/m³). The use of the same tip speed would have resulted in a stirring speed of 68 rpm (10 W/m³) in the 5 L vessel, a value that was too low to achieve a sufficient homogenization. The data establish that the DO could not be maintained at the set point of 40 % at a process time of ca. 170-195 h (Figure 4) at 100 rpm. Although the inlet gas mix was set at 100 % O₂, the flow rate had to be increased up to 20 sL/h in order to maintain the DO at 40 %. We observed that 140 rpm

optimized the DO signal throughout until the end of the process, keeping a steady 40 % DO (Figure 5) with a lower maximum flow rate till the end of the process. In this case, only about 16 sL/h were needed.

The results from the ring sparger vs. the L-sparger cell culture run showed a comparable performance of the two gassing systems (Figure 6). As in the $k_L a$ determination only a slight augmentation for the L-sparger should be noted for the use in the 5 L vessel. However, the differences in resulting growth and product formation were not significant (Figure 7). The cell growth and product concentration of both reactors were comparable to the 1 L small scale system. Both vessels can easily support a viable cell density of 2.5x10⁷ cells/mL within the defined fed-batch process range. However, further optimization of the process could be achieved by (a) adopting agitation as part of the DO cascade and (b) pursuing a more ideal optimization of the PID settings.

Conclusion

Managing scale-up and process transfer from development to manufacturing equipment is a challenging task. The ICH Q10 [3] states that “The goal of technology transfer activities is to move product and process knowledge between development and manufacturing, and within or between manufacturing sites to achieve an optimal final product. This knowledge forms the basis for the manufacturing process, control strategy, process validation approach, and ongoing continual improvement.” Herein, the process transfer must be performed in an organized and methodical manner, accompanied with appropriate documentation.

The scale-up and process transfer from the 1 L vessel of

the small scale DASGIP Parallel Bioprocess System to the 5 L vessel of the BioFlo 320 bench scale system can be achieved easily. The power number of the 1 L vessel is lower than the 5 L vessel for the single-stage system. However, it is comparable to the multi-stage stirred system. While using the tips speed as a scale-up criterion resulted in a visible lag for gas dispersion, the $k_L a$ value has proven to be an adequate scale-up measurement for the reviewed bioprocess in First CHOice Medium. The quality process parameters, the growth of the cells and the productivity of the bench top system showed a high level of comparability.

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Literature

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- [3] ICH Q10 Pharmaceutical Quality System. This document is designed to assist pharmaceutical manufacturers by describing a model for an effective quality management system for the pharmaceutical industry.

Ordering information

Description	Order no.
DASGIP® Parallel Bioreactor System , for cell culture, max. 50 sL/h gassing, 4-fold system with DASGIP® Bioblock	76DG04CCBB
DASGIP® Vessel , DS10000DSS, 350 mL – 1.0 L, 2x GL45 side arms, overhead drive, 2 pitched-blade impellers, DASGIP® Bioblock	76DS10000DSS
Rushton-Type Impeller , 6-blade, stainless steel, O.D. 46 mm, I.D. 8 mm	78100557
L-Sparger , D4 L300 W42 barb	78107298
BioFlo® 320 , base control station. All configured units include the same base control station.	1379963011
Vessel , for BioFlo® 320, water jacket, direct-drive, 5 L	M1379-1007
Pitched-Blade Impeller Kit , direct-drive, 5 L	M1379-1017
Rushton-Type Impeller , direct-drive only, 5 L	M1379-9297

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