The Flexibility of Small-Scale Single-Use Bioreactor Solutions

Christian Müller, Lena Nielsen, and Torben P. Frandsen

The authors present a case study in which four single-use vessels were fitted to an existing bioreactor system.

Disposable technologies are being used more frequently for the development and manufacture of complex biologics in the biopharmaceutical industry (1, 2). Small-scale (bench-top) single-use bioreactors (SUBs) provide a faster turnaround time through the elimination of cleaning, assembling, and autoclaving operations. Small-scale bioreactors are the workhorses for process development and optimization, and because they are also scale-down models for process characterization, it is important that these vessels, whether reusable or single-use, replicate the design of production-scale bioreactors. In the present study, the authors examined four different prest-erilized single-use vessels (**Table I**) that were fitted to an existing bioreactor system with minor modifications, and also compared the performance with a control glass bioreactor.

The single-use bioreactors are configured relatively similarly; some had more options for sparging (i.e., micro- or macro-spargers) or for use of single-use (pH and DO) sensors. The vessel height/diameter and the impeller diameter/vessel diameter are fairly similar for these units; however, the system from BioBlu (Eppendorf) is relatively wider and has a larger impeller. The UniVessel (Sartorius) and the Mobius (Merck Millipore) vessels are only available in one size, while the BioBlu exists in several versions ranging from 65 mL up to 40 L including packed-bed versions and furthermore with models for microbial applications. The CerCell bioreactors can be user-designed (i.e., variations in diameters, height, impellers/turbines, connections, single-use-sensor brands) using an online tool. The vessels can be designed ranging from 250 mL to 30 L in polycarbonate and also for microbial and packed-bed applications.

FITTING THE SUB TO THE EXISTING BIOREACOR SYSTEM

For the bioreactor evaluation, an existing control system (i.e., a BIOSTAT B-DCU II system from Sartorius) was used. This system's probes for temperature, pH, and dissolved oxygen (DO) could be used in all systems directly. For the stirrer, motor adaptors were obtained from the different vendors. The bioreactor temperature could in some cases be controlled using electrical heating blankets. Because this required modifications of the circuitry to the control system, it was decided to control the bioreactor temperature using the existing Biostat B-DCU II water-based thermostat system instead. This was done by attaching the thermostat system via Rectus quick connect couplings to Flexijacket (by Service-Tekniker.dk), which is a flexible black silicone water heating jacket that can be customized for the bioreactor size needed. Flexijackets with a length of 44.4 cm could be used for all the single-use bioreactors, and the temperature could be precisely controlled in the vessels.

TESTING THE SINGLE-USE BIOREACTORS

In this study, platform fed-batch cultivations were conducted using a Chinese hamster ovary (CHO) expression platform (CMC Biologics' CHEF-1) (3, 4) as a model system with a cell line expressing a model fusion protein. For the experiments, the four disposable bioreactors and a control 5-L glass bioreactor were run side-by-side on two separate occasions using the process parameters described in **Table II**. In all vessels, open hole or ring spargers were used. The

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Table I. Parameters and characteristics of the evaluated bench-top single-use bioreactors (mammalian cell culture).

Bioreactor	UniVessel SU	BioBlu 5c	CellVessel	Mobius CellReady	5L Glass
Vendor	Sartorius	Eppendorf	CerCell	Merck Millipore	Sartorius
System	Disposable	Disposable	Disposable	Disposable	Glass (control)
Total volume	2.6 L	5.0 L	3.0 L*	3 L	6.5L
Max. working volume	2.0 L	3.75 L	2.25 L*	2.4 L	5.2 L
Min. working volume	1.0 L	1.3 L	N/A	1.0 L	2.5 L
Impeller type	3-blade pitched impeller, 30° angle	3-blade pitched impeller, 45° angle	3-blade marine impeller*	3-blade marine impeller	3-blade pitched impeller, 45° angle
Number of impellers	2	1	1*	1	1
Impeller diameter	54 mm	100 mm	60 mm*	76.2 mm	68 mm
Vessel diameter (inner)	130 mm at top (1.5° slope)	170 mm	130 mm*	137 mm	158 mm
Impeller diameter/ vessel diameter	0.42	0.59	0.46	0.56	0.43
Vessel height	240 mm	256 mm	225 mm*	249 mm	335 mm
Vessel height/ diameter (H/D or aspect ratio)	1.8	1.5	1.7	1.8	2.1
Power Number, Np	1.2	1.3	N/A	0.3	N/A
Thermowell pocket for pt 100 temperature sensor	Yes, 8 mm	Yes, but narrows towards end (< 7.6 mm)	Yes, 8 mm*	Yes, 7.6 mm	Yes, 8 mm
Sparger type (used in this experiment underlined)	L-sparger	Porous Microsparger or macrosparger	L-sparger*	Sintered polyethylene microsparger or open pipe	Ring sparger
Sparger hole diameter	14 x 0.5 mm	7-12 µm or 0.7 mm open hole	10 x 750 µm holes*	15-30 µm pores or 2.3 mm hole	14 x 0.5 mm
pH probe	Single-use PreSens or reusable	Single-use PreSens or reusable	Single-use or reusable*	Reusable	Reusable
DO probe	Single-use PreSens or reusable	Reusable (into sleeve with permeable gas membrane)	Single-use or reusable*	Reusable	Re-usable
Can stirrer adaptors be supplied?	Yes	Yes	Yes	Yes	N/A
Comments	Requires SU connection box for use of single-use sensors; Torospherical bottom design	A 'slim' version of the vessel, BioBlu 3c, with an aspect ratio of 2.0 and the same max. working volume is also available. Single-use sensor requires connection box.	*Can be user customized/defined		Glass vessel used as control

single-use bioreactors have slightly different total volumes, working volumes, and stirrers; therefore, the initial working volume, overlay airflow, and stirring rates were adjusted to fit each bioreactor and to stir with similar power input per unit volume (W/m^3) (see **Table II**).

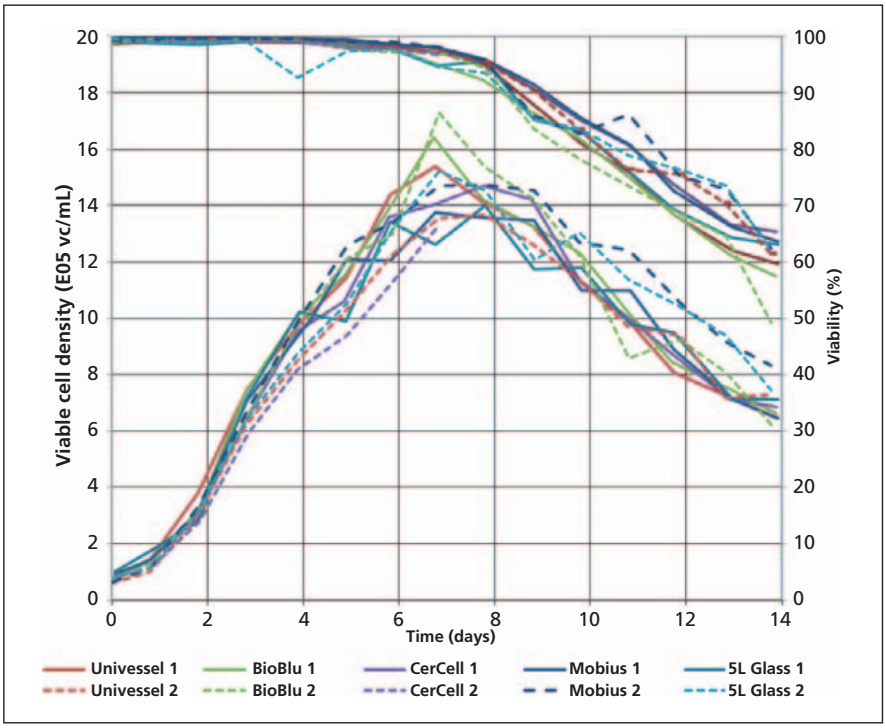
For investigation of the cell-culture performance, parameters including cell growth, viability, and metabolism and on-line trends for temperature, pH, and DO were compared. Samples were analyzed daily on a Cedex HiRes (Roche) and a Bioprofile 400 system (NOVA Biomedical), and after the cultivations were terminated, product concentration and key product quality characteristics were analyzed.

In each of the two experiments, fed-batch cultivations were conducted for 14 days in the four disposable bioreactors and the control glass bioreactor. As shown in **Figure 1**, viable cell density and cell viability were comparable during the course of the experiment for all bioreactors (single use and control) in both experiments.

Table II. Bioreactor process parameters.

Media			
Starting medium	CD-CIM1	Feed medium	Feeds A, B, and C
Set points, controls, and feeds			
Parameter	Set Point	Acid	CO_2 in overlay
Inoculation viable cell density	0.6E+06 viable cells/ml	Base	2M NaOH
Initial Temp.	37°C	Antifoam	ADCF Antifoam used if foam level is > 2 cm
First Temp. Shift	34°C from day 3	Second Temp. Shift	32°C from day 10
pH	6.90 (DB ±0.15)	Culture Duration	14 days
Dissolved oxygen	60% (optical probes)	O_2 on demand through the open hole/ring sparger is used	
Feed	Bolus feeds of 1.25%, 2% and 5% of initial bioreactor volume were given on days 3, 5, 7, 9 and 11		
Specific volumes, overlay, and stirring rates used			
Vessel	Initial working volume	Overlay airflow	Stirring rate
UniVessel SU (Sartorius)	1420 mL	90 mL/min	100 rpm
BioBlu (Eppendorf)	2660 mL	120 mL/min	80 rpm
CellVessel (CerCell)	1600 mL	90 mL/min	140 rpm
Mobius (Merck-Millipore)	1700 mL	90 mL/min	150 rpm
Glass vessel (Sartorius)	3680 mL	200 mL/min	150 rpm

Figure 1. Viable cell density and cell viability vs. cultivation time for the four disposable bioreactor runs compared to the control during 14 day fed-batch. Both Run 1 and Run 2 are depicted. Data were obtained daily from a Cedex HiRes (Roche).



Online trends of total CO_2 and oxygen usage, DO, temperature, and pH control for all bioreactors showed similar trends (data not shown). Comparable lactate, CO_2, and offline pH profiles were also obtained (**Figure 2**), indicating that the difference between the different single-use bioreactors and the glass bioreactor control is small, and that the cell-culture parameters are comparable even though the bioreactor set-ups are different.

Determination of the product titer (**Figure 3**) over the course of the two fed-batch experiments shows that the cells produce a comparable amount of product in the different bioreactor units. Furthermore, analysis of selected product characteristics (e.g., glycosylation, aggregation, and product-related variants) (data not shown) showed that the product is similar in the different bioreactors.

BIOREACTOR EASE-OF-USE EVALUATION

Generally, the use of single-use bioreactors is a major advantage for an efficient workflow during upstream process development and optimization because it can significantly reduce bioreactor down-time (or turnover time) by eliminating cleaning, assembling,

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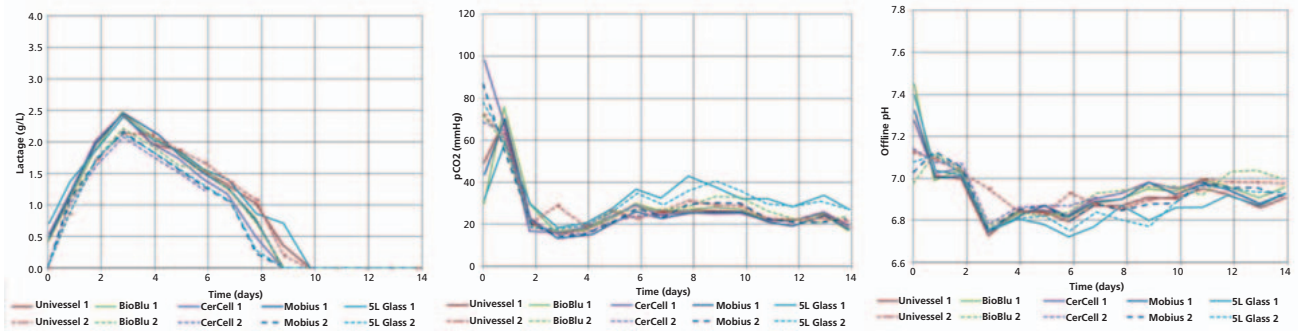
Figure 2. Lactate, pCO_2, and offline pH vs. cultivation time for the four disposable bioreactor runs compared to the control during 14 day fed-batch. Both Run 1 and Run 2 are depicted. Data were obtained using a Bioprofile 400 (Nova Medical) of offline pH-meter (Hamilton).



and autoclaving. Key operations such as pH and DO probe calibration and maintenance, media preparation, feed-tubing assembly, and sampling/analysis will still be needed—even if single-use pH or DO sensors are also implemented. The authors estimate, however, that use of these vessels can reduce the preparation time substantially.

During these experiments, a user evaluation was carried out examining the ease-of-use of the different single-use vessels for the CHEF-1 CHO platform process described. The amount of weldable connections were considered (3 feeds + glucose, antifoam, and base was in this case needed) as well as ease of sampling, how well condensate could be removed from the gas exhaust, amount of foaming, harvest options, and how well the stirrer adaptor worked.

The overall conclusion was that the four different single-use bioreactors tested all provided ample solutions for the examined fed-batch process. All bioreactors could be used as intended, and required little or no adjustment to be run effectively. Regarding feeding/sampling connections, all units provided weldable C-Flex connections (1/8 inch) for feed addition to the top of the bioreactor and some provided weldable C-Flex connections for addition to the bioreactor liquid. The BioBlu and UniVessel units also provided larger diameter (1/2 inch) weldable tube with Kleenpak Quick Connections, and the Mobius provided the large diameter (1/2 inch) weldable tubing connected to smaller weldable diameter (1/8 inch) tubing for flexibility. The CellVessel was user-designed with only the smaller 1/8-inch diameter weldable tubing due to the relative small volumes needed to feed/sample/harvest from the 2–5L bioreactors, and to minimize volume hold-up in the tubing. For sampling, aeration and agitation of the single-use bioreactors all worked similarly well despite the different solutions (e.g., regarding the stirrer adaptors). With respect to solutions for off-gas exhaust, the task of avoiding pressure

Figure 3. Product concentration vs. cultivation time for the four disposable bioreactor runs compared with the control during 14-day fed-batch. Both Run 1 and Run 2 are depicted. Data were obtained using a Protein A high-performance liquid chromatography (HPLC) method.

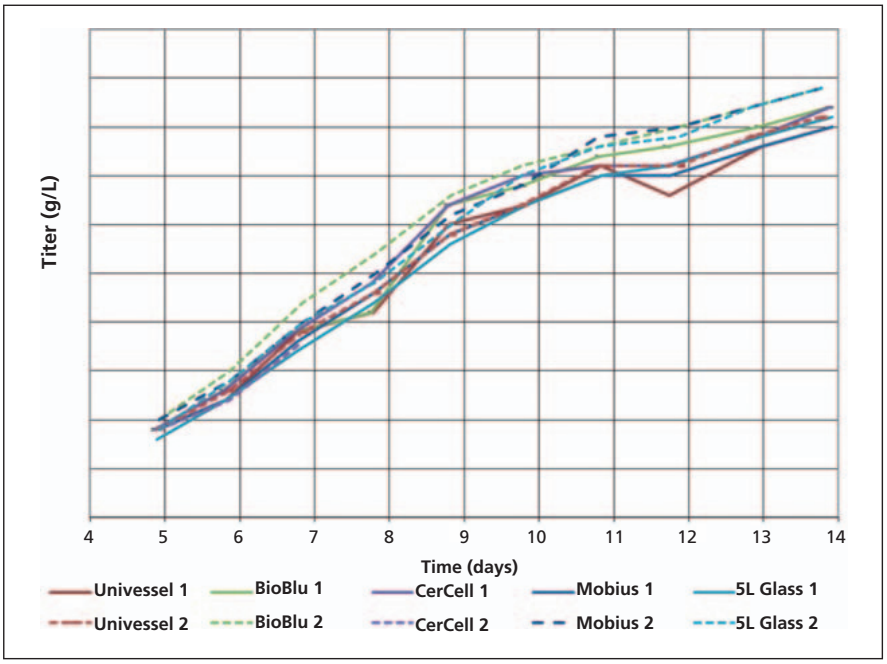
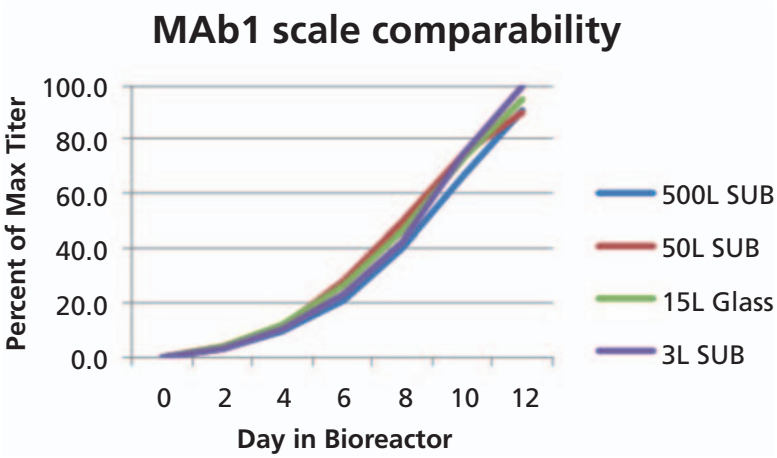


Figure 4. Product concentration vs. cultivation time for 3-L single-use bioreactor, 15-L glass vessels, 50-L, and 500-L single-use bioreactors for an antibody.



build-up in the bioreactor due do clogged air-filter was solved in different ways: UniVessel had two filters; BioBlu had a large off-gas filter (and possibility of Peltier cooling as an add-on); Mobius had a large-diameter tubing capable of removing condensate; and the CellVessel could be redesigned, if needed. All single-use bioreactor solutions for off-gas exhaust worked equally well in these experiments.

INTEGRATION BETWEEN MANUFACTURING SITES

Having standardized bioreactor solutions and designs is important both for small-scale (bench-top) and large-scale manufacturing applications when having multiple (global) manufacturing sites. Single-use bioreactors provide a standardized solution that can support a seamless transfer between sites when the same bioreactors are used at both ends. Additionally, eventual customization to bioreactor design can be used at all sites once produced at the supplier.

SCALE-UP AND CONCLUSION

An important consideration for implementing small-scale single-use bioreactors is scalability to large-scale bioreactors. This means the process can be developed and optimized prior to scale-up, and that troubleshooting or detailed process characterization campaigns, for example, can be carried out with a small-scale model that adequately represents the large-scale model.

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The small-scale bioreactor process examined was scaled up to 2000-L scale, and it was found that with respect to cell-culture performance, metabolite profiles, product titer, and product quality, both the single-use bioreactors and the 5-L glass vessels provided good small-scale models of the large-scale model. The exercise of comparing small-scale single-use bioreactors to small-scale glass vessels and large-scale bioreactors has been done in several projects. Another example is shown in **Figure 4**, where product concentration for a 3-L single-use bioreactor, 15-L glass vessels, 50-L, and 500-L single-use bioreactors was compared for a project with an antibody again demonstrating good scalability between the small scales (single use and glass bioreactors) and production scale.

CMC Biologics' experience with this technology has been generated over recent years (1, 2). A novel approach with implementation of multiple 2000L bioreactors in either 3Pack or 6Pack configurations has been introduced (5). The Bioreactor 6Pack configuration (e.g., consists of six 2000L production bioreactors and a 2000L seed train) allows for flexible production with scales from 2000–12,000L in a single production suite. The bioreactors can be run in single unit operations or in groups, simultaneously, sequentially, or in staggered fashion to achieve desired production needs.

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