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How Multipurpose is a Disposable Bioreactor?

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The authors discuss the use of single-use bioreactors throughout industry.

ABSTRACT

For almost 40 years, bioprocess engineers have been indoctrinated with the technology of the stirred tank bioreactor. But many have concluded that a plastic bag can be effective and that the results achieved with cultures in a single-use bioreactor are comparable with the results achieved in the glass or stainless-steel stirred tank bioreactor. This article describes experiments performed in the bag type single-use bioreactor that suggest it can yield results that are comparable with those as achieved in the traditional stirred tank bioreactor.

An increasing number of therapeutic candidates, including monoclonal antibodies, biotherapeutic proteins, and vaccines, are currently entering early-stage process development. At the same time, biologics are being introduced onto the market or have recently been introduced. In this competitive market, time-to-market, cost-effectiveness, and manufacturing flexibility are key issues that all must be achieved while maintaining product quality.

Traditionally stirred tanks, glass vessels, and stainless steel tanks have been used at laboratory and pilot scales for process development and production of research grade, toxicological, and Phase I clinical material. Stainless steel tanks dominate large-scale manufacture (> 1000L) of bio-therapeutics. However, fixed plant equipment is costly, requiring long lead times for installation and qualification. There is also a high burden from validation efforts related to sterility and cleaning, as well as for maintenance. The risk for cross contamination in standard steel or glass equipment leads to strict rules for cleaning and cleaning validation.

During the past decade, industry has been switching to disposables in medium preparation, storage of buffers, and even for cell culturing and downstream operations. For cell culture, various types of disposable technologies have been introduced, all with specific benefits and drawbacks.

Advanced cell-line engineering and process development have resulted in more productive cell cultures. During the past 15 years, cell culture titers in fed-batch processes have increased from 0.05 to over 10 gL⁻¹ today, allowing the use of smaller scale bioreactors.

Smaller bioreactors are gaining popularity, and this again has led to increased implementation of disposable technologies. While the industry is looking for high cell densities, high productivity, cost-effective process design, and speed to reach market introduction, the bioreactor demands are increasing, too. This is especially relevant for mixing and mass transfer but also for measuring and control of essential parameters, such as pH, dissolved oxygen (DO), glucose, lactate, and viable cell density.

High-cell density culture—including perfusion cultures—generate greater demands with regard to mixing and mass transfer. Microbial fermentation processes are even more demanding in terms of mass transfer when compared with a cell culture process. The currently available disposable bioreactor systems are less suitable for high-density cell culture processes and are not suitable for microbial fermentation processes. The exception here is the CELL-tainer bioreactor (CeLLution Biotech, Assen Netherlands). The performance of this bioreactor is comparable with the stirred tank and thus covers the complete range of applications from cell culture to microbial fermentations, from adherent cell cultures to more viscous fungal fermentations. This article will discuss a rocking-based disposable bioreactor that can be used in a wide range of biotechnological processes.

TYPES OF SINGLE USE BIOREACTORS WITH DISPOSABLE BAGS

One of the keys to properly using single-use bioreactors is the application of disposable bags available with or without integrated sensors and equipped with connections for feed, inoculums, sampling, and with gas inlet and exhaust gas filters. These bags are pre-sterilized using gamma irradiation, ensuring full sterility.

One of the challenges for using these presterilized bags is to ensure proper mixing, mass- and heat transfer, and proper process measurement and control. This all should be comparable with traditional stirred bioreactors.

To prevent oxygen limitation, a high-demanding cell-culture process requires an oxygen mass transfer capacity of 10 mmol/L.hr when 50 x 10⁶ cells/mL are cultivated. This translates to a required $k_La = 50 \text{ hr}^{-1}$. For microbial systems, like an *E. coli* fermentation at 50 g/L dry cell weight, the required mass transfer capacity has to be 200 mmol/L.hr or even higher, meaning a $k_La > 800 \text{ hr}^{-1}$ using air.

Table II: Overview of commercially available disposable bioreactors.

Bioreactor Type	Working Volume (L)	Type of Bag	Type of Mixing	Supplier	Ref.
Wave Bioreactor	1-100 L	flexible bag	rocking	GE Healthcare	[1, 2]
Corning Mill	1-100 L	flexible bag	rocking	Corning System Biotech	[1, 3]
MapleBio	1-100 L	flexible bag	rocking	Maple BioScience	[1, 4]
CellCulture	1-100 L	flexible bag	rocking	CellCulture Biotech	[1, 5]
Corning CDMO	10-1000 L	flexible bag	stirred	Corning System Biotech	[1, 6]
Wave	10-1000 L	flexible bag	stirred	Thermo Fisher Scientific	[1, 7]
Maple	10-1000 L	flexible bag	stirred	MapleBio	[1, 8]
Maple	10-1000 L	flexible bag	stirred	Maple BioScience	[1, 9]
Maple	10-1000 L	flexible bag	stirred	Maple BioScience	[1, 10]
Maple	10-1000 L	flexible bag	stirred	Maple BioScience	[1, 11]
Maple	10-1000 L	flexible bag	stirred	Maple BioScience	[1, 12]
Maple	10-1000 L	flexible bag	stirred	Maple BioScience	[1, 13]
Maple	10-1000 L	flexible bag	stirred	Maple BioScience	[1, 14]
Maple	10-1000 L	flexible bag	stirred	Maple BioScience	[1, 15]
Maple	10-1000 L	flexible bag	stirred	Maple BioScience	[1, 16]
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Maple	10-1000 L	flexible bag	stirred	Maple BioScience	[1, 46]
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Maple	10-1000 L	flexible bag	stirred	Maple BioScience	[1, 48]
Maple	10-1000 L	flexible bag	stirred	Maple BioScience	[1, 49]
Maple	10-1000 L	flexible bag	stirred	Maple BioScience	[1, 50]

*k_La = volumetric oxygen transfer coefficient (per liter per hour)

Table I: Overview of commercially available disposable bioreactors.

The rocking type bioreactor ensures easy operation due to its simple construction and handling. Mixing profiles might be different from stirred bioreactors; however, as the microenvironment of the cell determines the physiological status of the cell, the cells seem to do fine as long as there are no gradients of temperature, oxygen, or CO₂. When the presence of nutrients is assured and the pH is acceptable, the cells "do not care" in what type of bioreactor they are cultured. The challenge is to ensure proper micromixing and mass transfer and to avoid gradients.

The Wave Bioreactor

Single-use bioreactors are commercially available at various scales from the laboratory scale and pilot scale (1-100 L) up to even production scale (1000 to 2000 L). The application, however, is primarily restricted to mammalian cell culture processes. (see Table I.)

Rocking type bioreactors

The Wave Bioreactor consists of a disposable bag, which contains the cells and media and is placed on a heated rocker (Figure 1) (1). Headspace aeration is used to inflate the "cellbag", and a rocking motion should create mixing. Gas liquid mass transfer occurs via the liquid-gas surface.



Figure 1. Wave Bioreactor example. (COURTESY: GENENTECH INC.)

The k_{La} is 10 - 30 hr^{-1} as reported by various authors (2). Mixing times are in the order of magnitude of 2 to 3 minutes for scales up to 100 L. The mixing time increases up to 5 minutes, especially at lower rocking speeds (< 20 rpm) or at larger scales (> 100 L). From a simple regime analysis, one may conclude that the Wave type bioreactors are working in a so-called mixed regime where mixing time and mass transfer are in the same order of magnitude, possibly leading to gradients of oxygen and CO_2 . Scalability of this system is not obvious as demonstrated by Eibl and Eibl (3). The literature suggests that the Wave Bioreactor is not suited for mimicking the cultivation conditions of stirred bioreactors for microbial culture conditions.

A number of similar wave-like systems, operating on similar principles, have been introduced, including those from Sartorius (Cultibag) and Applikon (AppliFlex).

UNDERSTANDING THE CELL-TAINER BIOREACTOR



Figure 2. CELL-tainer single use bioreactor. (COURTESY: HAN BIOCENTRE)

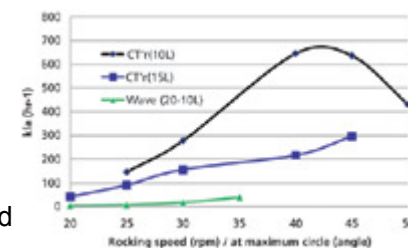
The CELL-tainer technology (see Figure 2) is based on a 2D rocking motion and application of a pillow-shaped or rectangular three-dimensional bag. Due to the two-dimensional rocking motion (in vertical and horizontal direction at the same time), the mass transfer is much higher when compared with other rocking systems (4).

High mass transfer has the potential of supporting higher cell densities, better stripping capacity of CO_2 , cell culture application as well as application in microbial and fungal fermentation. Besides improvement of the mass transfer, this bioreactor offers a removable segmentation of the bags, which makes it possible to start at volumes as low as 200-250mL and expand the culture in one-and-the-same bag up to working volumes of 15 L. The sensors, including an electrochemical sensor for pH and a polarographic sensor for DO both in a disposable format are mounted in the bottom of the bag and positioned in small cups, which guarantees proper process control even with low volumes under shaking conditions. Because traditional sensor technology is used, the range of measurement for pH is not restricted (pH: 0 to 14) as it is with optical sensors (pH: 6.5 to 8.0).

Temperature control of the bioreactor bag is located inside the incubator cabinet and by convection. No heating blanket is applied. When heat is generated by the culture, (e.g., with microbial fermentation, cooling is required). The CELL-tainer is equipped with an integrated cooling plate in the rocking platform. Using a temperature difference of 25 $^{\circ}\text{C}$, the cooling capacity is 500W, which is sufficient for a high density *E. coli* culture.

Investigation of the mass transfer, shows that the CELL-tainer covers a wide range of mass transfer values (see Figure 3). This is far beyond the capabilities of the traditional rocking type of bioreactors.

In most mammalian cultures, the mass transfer for oxygen seems to be sufficient to support high cell densities, at least in the stirred bioreactors. To enhance oxygen transfer, stirred bioreactor (micro-) spargers are applied and air may be enriched with oxygen. In both stirred and wave type single-use bioreactors, the exchange of CO_2 might be limited due to a lower mass transfer coefficient and



due to limitations in stripping efficiency. As the mass transfer coefficient in the CELL-tainer bioreactor is much higher than in wave type and stirred single-use bioreactors, the liquid phase CO₂ concentration is always in equilibrium with the gas phase CO₂ concentration. This results in less CO₂ build-up in the liquid phase, which results in reduced alkaline addition, and which benefits the culture as a whole.

Figure 3. Mass transfer in a CELL-tainer bioreactor (tap water, 20 Å°C) (Data CELLution Biotech) compared to the Wave Bioreactor.

The mass transfer that can be achieved in the CELL-tainer bioreactor is significantly higher ($k_{l}a > 300 \text{ hr}^{-1}$) than that seen in the wave type bioreactors and that therefore opens the application of single-use equipment for microbial fermentations as well.

MICROBIAL FERMENTATION IN THE CELL-TAINER BIOREACTOR

Interest in microbial expression systems such as *E. coli* and *Pichia pastoris*, is increasing. That's the case not only for traditional products such as enzymes but also for production of biopharmaceuticals and the manufacturing of platform chemicals. Bulk fermentation products are produced on large scales of up to 400 Å 800 m³ in stirred bioreactors, but for screening and pre-culture purposes, the single-use bioreactors offer the advantage of fast turnaround, speed in early stage development, and late stage development (scale-down experiments). The limited infrastructure required (no autoclaves or SIP/CIP) is also a key advantage for single-use equipment.

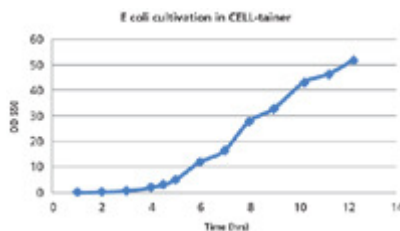


Figure 4. Cultivation of *E. coli* in a 10 L fed-batch in the CELL-tainer bioreactor. OD550 = biomass concentration in optical density units measured at 550 nm.

Figure 4 shows microbial culture in a 10L fed-batch performed in the CELL-tainer single-use bioreactor. The profile shows results that are comparable with those seen in stirred fermentors up to 100 L working volumes (5).

In addition to the pH and DO sensor, a device for measuring glucose and lactate is available, using the Trace Analytics (www.trace.de) technology of a dialysis membrane. The dialysis membrane is integrated in the bottom of the bag, comparable to the pH and DO sensors. The device is an integrated part of the gamma-irradiated bag (see figure 5).

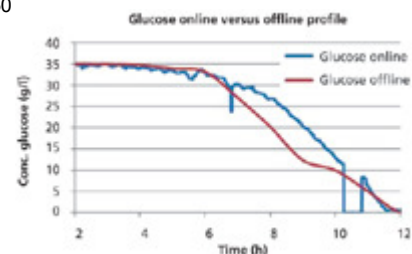


Figure 6. Comparison of in-line and on-line glucose measurement in an *E. coli* culture using the ContiTRACE dialysis membrane plug integrated in the CELL-tainer.

In an *E. coli* fermentation, using glucose as substrate, online and offline data are compared (see figure 6). There is only a slight, negligible discrepancy between on-line and off-line data. The off-line data are based on an enzymatic method in collected samples using an autosampler. The discrepancy is easy to explain: the off-line data show lower values because of the metabolic activity that continues after a sample till analysis takes place (2Å5 minutes before the sample is Å5 Å°C). The online measurements are highly accurate because there is continuous equilibrium between the culture and the cell-free dialysate. The ability of online measurement of an important parameter such as glucose (and lactate) offers the possibility to develop advanced on-line control strategies, thus making the single-use bioreactor increasingly suitable for process development.



Figure 5. Single-use dialysis membrane. (COURTESY: TRACE ANALYTICS GMBH/CELLUTION BIOTECH BV.)

COST COMPARISON

Single-use bioreactors offer the advantage of fast installation, lower investments in infrastructure, and a significant decrease of validation costs. Similarly a comparison of operational costs of a 5-day microbial process as performed in a 15L autoclavable, in a 15L SIP bioreactor, versus the process in the single-use CELL-tainer, reveals significantly lower costs in the single-use system per run (see figure 7). This includes the costs of bags (fully equipped with sensors). Because the single use CELL-tainer bioreactor has a fast turnaround time, in the same bioreactor system, 40% more runs/year can be made, when compared to an autoclavable fermenter.

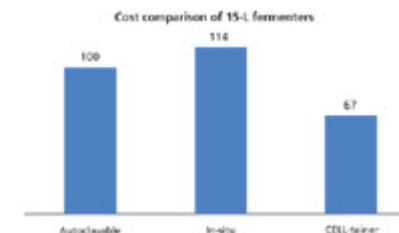


Figure 7. Relative operational cost comparison of 15-L bioreactors of different types.

CONCLUSION

Single-use bioreactors have become accepted by industry and academic laboratories because of ease of use, flexibility, cost of operation, and lower investments. The CELL-tainer single-use bioreactor system can be used in a wide variety of applications: intensive cell cultures, fragile cultivation systems like with micro-carriers and hybridoma cultures, and also in microbial fermentations (e.g. yeast and fungal cultures) due to its ability to deliver proper mixing and high mass transfer. Because the system is equipped with advanced process control, which could include in-line analysis of glucose and lactate, the CELL-tainer widens the application of single-use bioreactors even to traditional biotechnology processes as a process development tool or as pre-culture system.

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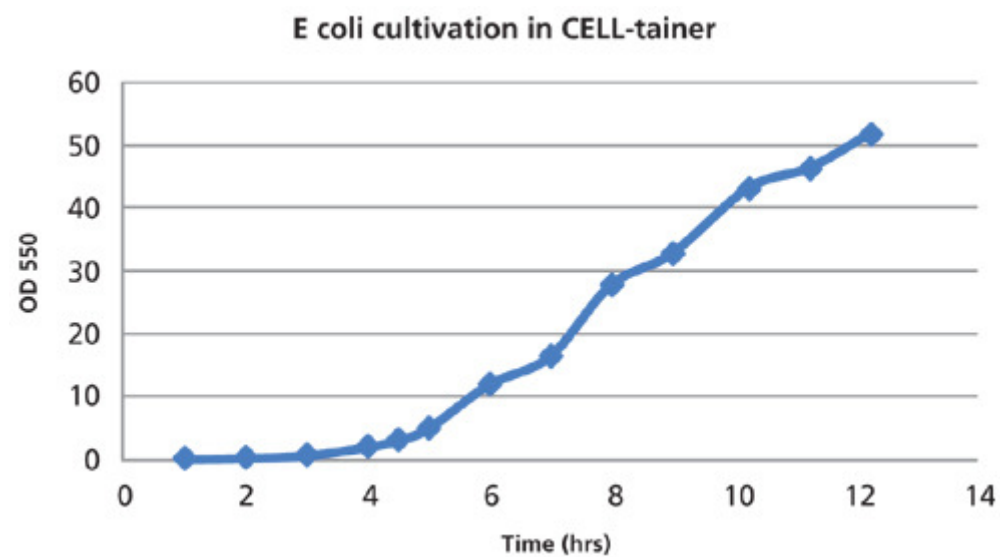
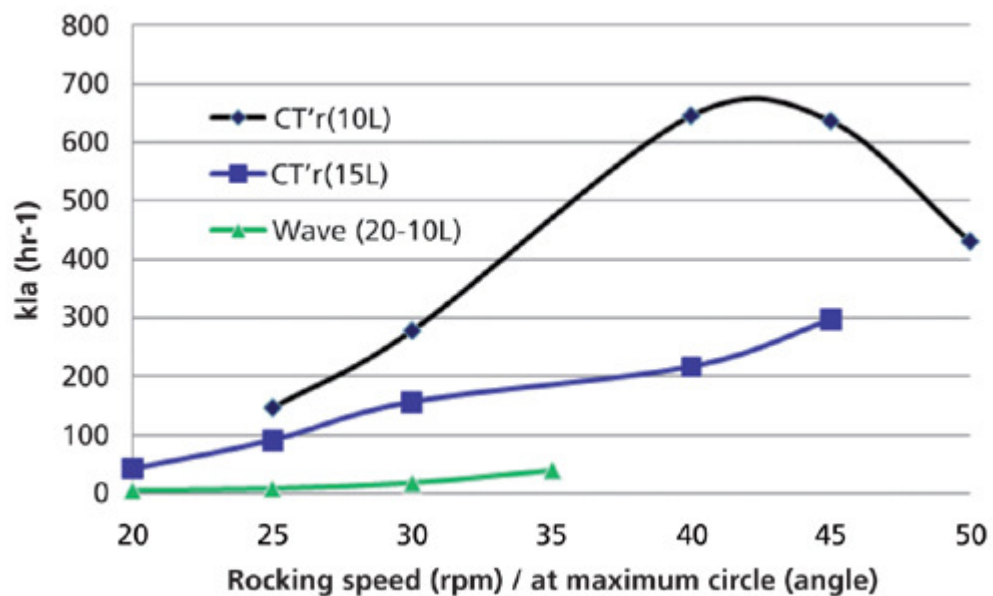
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Reactor type	Working volume	Type of bag	Type of mixing (hr ⁻¹)	Supplier	k _a * (hr ⁻¹)
Wave Bioreactor	1 – 200 L	pillow shape	rocking	GE Healthcare	< 30
Cultibag RM	1 – 100 L	pillow shape	rocking	Sartorius Stedim Biotech	< 30
AppliFlex	1 – 25 L	pillow shape	rocking	Applikon Biotechnology	< 40
CELL-tainer	0,2 – 25 L	pillow shape or rectangular 3 D	rocking in two directions (2D)	CELLution Biotech	>300
Cultibag STR200	50 – 200 L	tankliner	stirred	Sartorius Stedim Biotech	< 40
SUB	50 – 1000 L	tankliner	stirred	Thermo-Fischer (Hyclone)	< 40
XDR	40 – 2000 L	tankliner	stirred	XCellerex	< 20
Nucleo	50 – 100 L	square 3D	paddle agitation	ATMI / Pierre Guerin	< 20
Shaking bioreactor	< 200 L	tankliner	orbital shaker	Kühner/ExcellGene	<10
CellMaker Regular	1 – 50 L	bubble column	rotating sparger	Cellexus	< 10

*K_a = volumetric oxygen transfer coefficient (expressed as hr⁻¹)



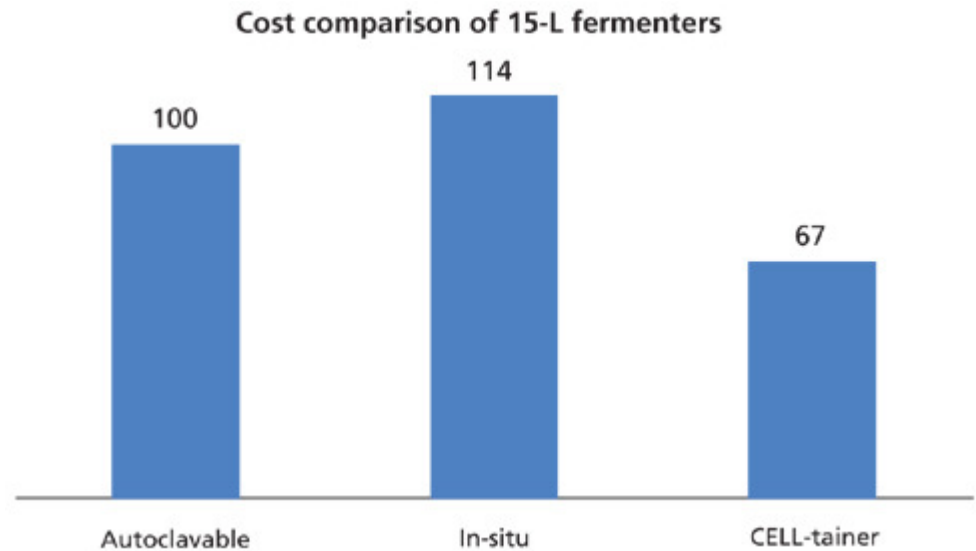
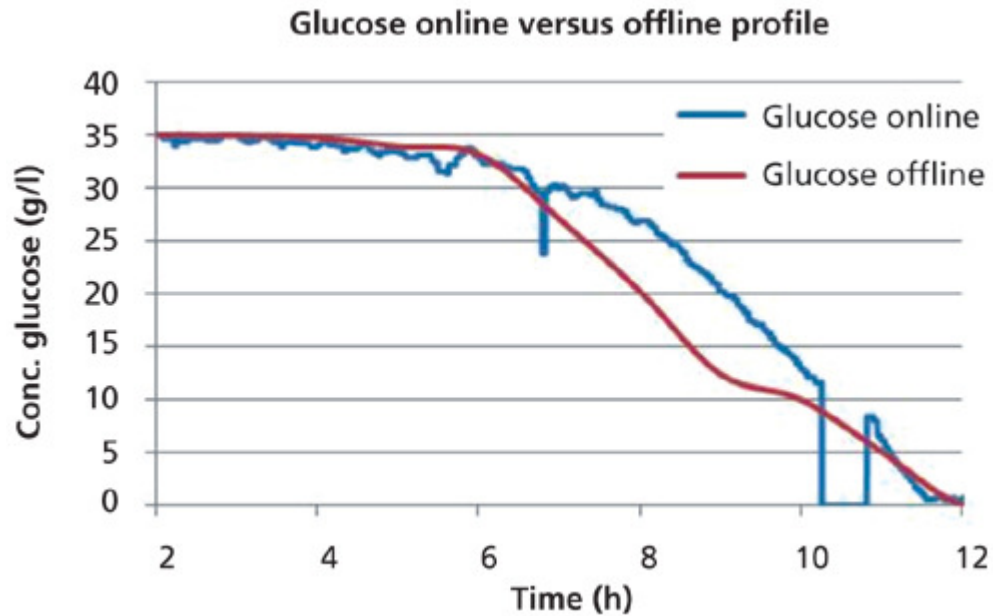


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