Large-Scale Disposable Shaking Bioreactors

A Promising Choice

Keyur Raval, Chao-Min Liu, and Jochen Büchs

As disposables become increasingly important in biopharmaceutical manufacturing, more companies are replacing rigid stainless steel and glass components with flexible, single-use plastics. Mixing tanks, filter assemblies, and tubing are some types of components that have successfully been replaced by disposable elements for cell-culture production of high-value compounds. However, the Wave bioreactor (www.wavebiotech.com) is so far the only available disposable option to conventional bioreactors. Here, we present a disposable bioreactor concept based on shake mixing, properties of which are outlined in the "Advantages" box.

The Reactor Assembly

As shown in Photo 1, cylindrical vessels (Nalgene, USA; www.nalgene.com) of 20 L and 50 L are mounted on a standard RC-6 shaking machine from Kühner AG (Switzerland; www.kuhner.com). Vessels are made of polypropylene or from transparent polycarbonate. The machine has a maximum loading capacity of 200 kg, with shaking frequencies from 20 to 400 rpm. Each vessel has a large-diameter closure (not mounted on the right vessel in Photo 1) to ensure easy mounting of disposable air inlet, outlet, and sample ports. A normal electrochemical dissolved oxygen electrode can be mounted on the closure. Use of optical sensors for pH and dissolved oxygen (PreSens GmbH, Germany; www.presens.de/html/start.html) can leave more space on the closure.

Photo 1: 50-L (left) and 20-L bioreactors mounted on a standard RC-6 shaking machine (Kühner AG, Switzerland). The shaking machine has a fixed shaking diameter of 50 mm.

Mixing Performance

Cell culture systems are sensitive to concentration, pH, and temperature gradients. These gradients may appear because of nonhomogeneous or generally poor mixing. Bioreactors used for animal and plant cell cultures must therefore possess good mixing characteristics without generating large hydromechanical stresses.

The mixing performance of a disposable shaking reactor was measured by an electrical conductivity method. A tracer of 0.5 mL of 1M sodium chloride was added to the vessel at steady state, after which the electrical conductivity of the liquid was measured. The mixing time was defined as that for 99% of the total change in concentration following addition of the tracer. Figure 1 shows the mixing time of 20-L and 50-L vessels at 15-L and 35-L working volumes, respectively. As the figure depicts, for shaking frequencies larger than 80 rpm, mixing occurs only a few seconds after addition of the tracer. Although mixing also occurs at frequencies lower than 80 rpm, it is not recommended to operate under these conditions because no regular liquid flow pattern is observed (1).

Power consumption is an important characteristic of a reactor's performance. The magnitude of hydromechanical stress depends on the magnitude of power consumption. The power consumption in 20-L and 50-L vessels was measured using the temperature method developed by Sumino et al. (2). Results show that the order of magnitude of power consumption obtained is almost the same as that obtained in small shake flasks (3). Power consumption is more evenly distributed in shake mixing than in stirred mixing (4), thus resulting in very low levels of hydromechanical stress. Oxygen transfer and heat removal are also

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Process Focus: Cell culture and fermentation

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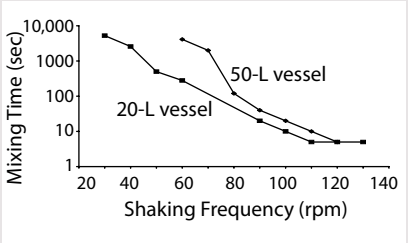
Level: Introductory

Figure 1: Mixing time of 20-L and 50-L disposable shaking bioreactors with 15-L and 35-L filling volumes, respectively. Experiments were performed at different shaking frequencies, with a 50-mm shaking diameter.



important parameters of bioreactor performance (**3**). The oxygen transfer in a shaken vessel is generally sufficient for growth of plant and animal cells.

Applications

Liu et al. were the first to scale-up production processes based on the animal and insect cell lines in disposable shaking bioreactors (**5**). They successfully cultivated hybridoma cells, Chinese hamster ovary (CHO) cells, and insect cell lines Sf-9 and H-5 (which demand higher oxygen rates than mammalian cells). They began with a normal batch operation, then moved to fed-batch, semicontinuous, and continuous operations, using cylindrical disposable reactors ranging from 3 L to 50 L (**6**). In all the cases, cell growth was better than that obtained by spinner flasks or a standard fermentor. Sf-9 cells were cultivated to a maximum viable cell density of >1 × 10^7 cells/mL in 4-L and 20-L bioreactors. Similarly, H-5 cells were grown successfully in a 20-L shaking bioreactor to a viable cell density of 5 × 10^6 cells/mL for scale-up production of recombinant proteins using a baculovirus/H-5 cell expression system.

Liu et al. also evaluated IgG production using hybridoma cells in shaking bioreactors from 3 L to 50 L in semicontinuous mode. The experiments were conducted with 11% exchange/day of culture broth with fresh medium. IgG production reached 150 mg/L per day while maintaining 2 × 10^6 viable cells/mL. A maximum of 250 mg/L of IgG was produced in the same process after termination of the daily exchange of broth and medium. CHO cells were grown in a fed-batch mode in 50-L shaking bioreactor with a maximum viable cell count of 6 × 10^6 cells/mL.

Liu's group routinely uses 20-L scale shaking bioreactors with working volumes of 5–10 L to grow suspension-adapted mammalian (e.g., CHO, HEK293, hybridoma) and insect cells for recombinant protein expression and live cell production to support high throughput drug screening programs (Photos 2–4). Despite their successful cultivation of different cell cultures, the authors stressed the need for further characterization of these simple-though-efficient shaking bioreactors.

Initial characterization experiments based on oxygen transfer revealed that not only slow-growing mammalian and insect cells but relatively fast-growing plant cells can be easily cultivated. To prove this, one of the fastest growing plant cell cultures, transgenic BY2 tobacco cells (*Nicotiana tabacum* L. cv. Bright Yellow 2), were successfully scaled-up in a 20-L bioreactor with 10-L filling volume (**7**). We used a transgenic version of these plant cells producing human serum albumin (HSA) (**6**), cultivating them with standard Gamborg's B5 plant cell culture medium. HSA was determined by ELISA. Figure 2 compares the growth profiles in a 250-mL shake flask and a 20-L disposable shaking

Advantages

Use of shake mixing in almost all biotech research laboratories makes cylindrical disposable shaking bioreactors easy to use, requiring no special training. Other favorable properties include

Well defined gas/liquid mass transfer area (**9, 10**)

Negligible foaming (**10**)

Ease of characterization using existing methods

Low levels of hydromechanical stresses due to homogeneous distribution of the power consumption (**4**)

Use of standard vessels and shaking machines that reduce initial costs (no need to purchase special bags and rocking machines)

Figure 2: Growth profile and HSA production of *N. tabacum* L. CV BY2 cells in a 250-mL shake flask with 150-mL filling volume and in a 20-L bioreactor with 10-L filling volume, 0.1 vvm, 180 rpm, 50-mm shaking diameter.

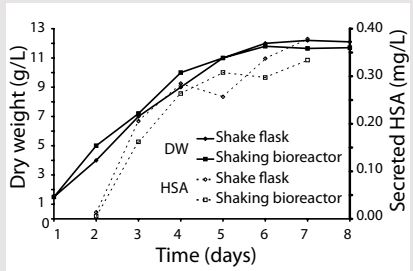


Photo 2: Insect cells (Sf-9) cultured in 20-L polyprolylene bioreactors, with a 7-L culture volume

Photo 3: CHO cells cultured in 20-L polycarbonate bioreactors, with a 5-L culture volume

Photo 4: Hybridoma cells cultured in a 50-L polypropylene bioreactor, with a 36-L culture volume (see reference 7 for details)

bioreactor. Aeration was kept at 0.1 vvm (volume of air per volume of medium per minute) in the disposable shaking bioreactor. It should be noted that this aeration value resulted in the same headspace gas concentration as that obtained in a 250-mL shake flask with a cotton plug in which all preliminary experiments were performed. The aeration value in that shake flask was obtained from the method developed by Mrotzek et al. (**8**). The cell growth profiles were identical in the 250-mL shake flask and the large disposable shaking bioreactor (Figure 2).

HSA production is a growth-associated process. Its production profile is also shown in Figure 2. As the figure depicts, no major difference was observed in HSA production during scale-up. Further, this HSA production process was successfully scaled-up in a 50-L bioreactor with a 35-L filling volume (results not shown). In a different experiment, growth kinetics with the same cell line were compared from a 20-L shaken vessel and a 7-L stirred tank and found to be similar. The *N. tabacum* L.CV BY2 cell line is relatively tolerant of hydromechanical stress, but as growth proceeds, foam generation can limit the stirring speed and aeration in a stirred-tank bioreactor. On the other hand, we have seen negligible foaming in a shaking bioreactor.

Large Scale Shaking Technology

Our findings show that shaking technology is not limited only to milliliter scale. The maximum reactor size described in this article is 50 L, although the maximum permissible size of disposable shaking bioreactors is not yet known. That maximum size/volume may be determined by the limitations of shaking machines, the generation of higher levels of hydromechanical stress with increasing reactor volumes, the amount of heat transfer through the reactor wall, oxygen transfer, or space availability. We continue to evaluate those parameters, but thus far find the maximum reactor volume applicable to animal- and plant-cell culture processes.

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