Shaken Bioreactors Provide Culture Alternative

Systems Have Evolved to the Point Where Scale-Up Is No Longer an Issue

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The application of shaken bioreactors for cell cultivation has increased enormously in the last eight years. These bioreactors are used for the expression (transient and stable) of recombinant proteins in drug and diagnostic research. Extensive tests demonstrate their potential in terms of performance, flexibility, and cost-effectiveness when compared with more conventional technologies.

Shaken bioreactors are typically operated in a temperature-, CO_2-, and humidity-controlled environment. For medium-scale work with volumes from 50 mL up to 1 L of culture medium, disposable Erlenmeyer flasks, both with small bottom baffles and without baffles, are most commonly used.

For smaller scale work, the use of microtiter plates (12, 24, 48, and 96 well) has been established. Here evaporation is the most important issue; this can be partially solved using a humidified environment and special plastic membrane lids for the microtiter plates.

Special centrifuge tubes, so called TubeSpin® bioreactors (**Techno Plastic Products**), with a working volume from 5 mL to 35 mL, have a ventilation cap and cover the range between the small- and the medium-scale work. Compared to the spinner flask, shaking technology has two major advantages—a single motor can provide shaking activity to numerous bioreactors, and there are no complicated or cost intensive moving parts in the shaken bioreactor, while providing very efficient mixing. Both spinner and shaker systems do have one important parameter in common: low shear stress.

Ten years ago, C.M. Liu, a Roche researcher who sought to capitalize on the low shear stress benefit, developed a simple system in which cell cultivation was carried out in 50 L cylindrical vessels on an orbital shaker. The 50 L vessels had a working volume of 36 L, and the head space was actively aerated by a mixed gas.

Inspired by this simple and successful idea, Jochen Büchs, professor of biochemical engineering at RWTH Aachen University conducted research using the same vessel size. Mixing time and heat transfer were characterized. Professor Büchs' team then performed parallel cultivations in shaken and stirred bioreactors with plant cells, which showed closely matching results.

Subsequently, Professor Florian Wurm, professor at EPFL and founder and CSO at **ExcellGene** (www.excellgene.com), and **Kühner** (www.kuhner.com) began to develop the next generation of shaken bioreactors for volumes up to 200 L. Using an orbital shaken bioreactor system rather than existing bioreactor systems, offers several advantages.

The orbital shaken bioreactor (SB200-X) is designed without baffles and aeration through the head space means no breaking bubbles and little foam formation. Together these features deliver low shear stress since the bulk of the liquid is exposed to laminar flow only. The disposable bag for the shaken system is simple in design and also cost effective because, unlike the stirred disposable system, there are no moving parts (stirrer) involved.

The ability to scale-up is easier with the shaken bioreactor as the fluid hydrodynamics are comparable from microliter volumes all the way up to 200 L. In addition, the system provides flexibility by covering a wide range of working volumes from 50–200 L.

Figure 1. SB200-X orbital shaken bioreactor

Pilot Shaker

The basic set up of the disposable shaken bioreactor system is shown in *Figure 1*. The shaker is based on the Kuhner Pilot Shaker. The vessel is made of stainless steel. A specially designed bag from **Sartorius Stedim Biotech**, is placed inside the vessel. This bag has a total volume of 310 L and includes three ports. The top ports are for exhaust air. The side ports measure temperature, pH, and DO. A sampling port is also available on the side. The bottom port provides drainage.

pH and DO are measured online by an optical measuring method (**PreSens**). The DO range is from 0–100%, and the pH range is 5.5 to 8.5. The culture medium is cooled down and heated through the bottom of the vessel.

In collaboration with ExcellGene, extensive studies to determine mixing times and kLa values were carried out with this system. Cell culture comparisons between different cultivation systems were also completed.

Figure 2 illustrates mixing time using a working volume of 100 L. As the graph shows, the mixing time depends on the shaking frequency—the higher the shaking frequency, the lower the mixing time.

ExcellGene carried out cell cultivation at a shaking frequency of about 60 rpm (with a shaking diameter of 50 mm). This resulted in a mixing time of about 30 seconds. Even with a working volume of 150 L and a shaking speed of 65 rpm, the same mixing time is achieved. A comparison with Professor Büchs's team working with 50 L cylindrical shaken bioreactors shows the same range and behavior of mixing times, using a different method.

Figure 2 shows results of the determination of the kLa values at 100 L working volume. At lower shaking frequencies, the kLa value is around 5 1/h. By increasing the shaking speed, the kLa rises to 25 1/h. This kLa range compares well with measurements carried out in shaken flasks.

ExcellGene also conducted a series of experiment to demonstrate the ease of scale-up with the new 200 L shaken bioreactor system. The cell line CHODG44 (medium proCHO5 supplemented with 4mM Glutamine) was cultivated in four different bioreactor systems:

1. TubeSpin bioreactors (shaken): 50 mL bioreactor with 10 mL working volume,
2. Round glass bottle (shaken): 5 L bioreactor with 1.5 L working volume,
3. Stirred bioreactor: 3 L bioreactor with 2 L working volume, and
4. SB200-X (shaken): 310 L bioreactor with 40 L and 200 L working volumes.

The data for 40 L working volume in the SB200-X is similar to that for the 200 L working volume, which is shown in *Figure 3*. All the data shows comparable growth curves even though the volumes range from 20 mL to 200 L (over four decades) and two different systems, stirred and shaken, were used. During both cultivations the DO probes showed no oxygen limitation in the 200 L disposable shaking system. These experiments give an impressive visualization of the ability to scale-up using the new SB200-X shaken bioreactor system.

The shaken bioreactor should be considered an alternative to disposable bag systems in the 200 L range. The data shown, demonstrates how easy it is to scale-up, while low shear stress and easy handling are known advantages of shaken bioreactors. Further scale-up is already being considered and initial successful experiments have been carried out with a 1,000 L shaken bioreactor prototype. GEN

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Figure 2. Mixing time and kLa-value versus shaking frequency in the SB200-X (100 L working volume)

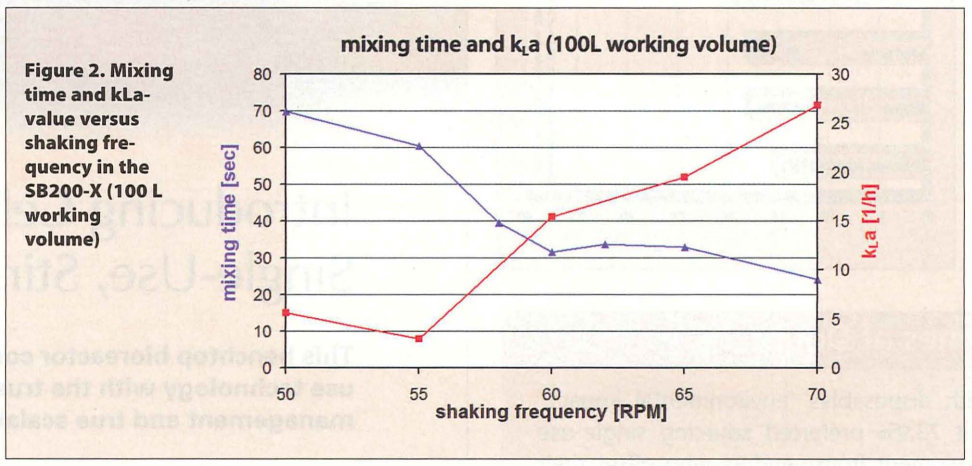

Figure 3. Cell count versus time in different bioreactor systems (SB200-X with 200 L working volume)