Update

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Bioprocessing Systems Go Disposable

Single-use disposable technology is gaining acceptance in the biopharmaceutical arena, especially with processors seeking to reduce their operating costs and add flexibility to their manufacturing schemes. "For bioreactors, the initial investment cost with the disposable systems — at clinical manufacturing (200–2,000 L) and commercial manufacturing (>2,000L) scales — is approximately half the costs associated with conventional bioreactors," says Robert Dream, director of pharmaceutical/biotechnology, Europe, for CH2M HILL Lockwood Greene (www.lg.com).

In terms of safety, disposable bioreactors greatly reduce the risk of cross-contamination, which is crucial for the applications in which this technology is gaining ground, namely: cell therapy (human cells for the treatment of cancer and immune system diseases); production of monoclonal antibodies (mAb) and antigens (using hybridoma cell-line diagnostic test kits and micro arrays); biopharmaceuticals and cytokines; and vaccines (human and veterinary).

Over the last year, the trend towards disposable bioprocessing has become more distinct, due to the regular achievement of milestones. In March, the biopharmaceutical company Bavarian Nordic A/S www.bavarian-nordic.com) reported the successful implementation of Wave Biotech LLC's (Somerset, NJ; www.wavebiotech.com) disposable Wave Bioreactor technology for the production of vaccines at its Kvistgaard, Denmark, plant.

The Wave Bioreactor was installed and commissioned in about three months — saving 6–9 months of time — which was essential to Bavarian Nordic because of the participation in the U.S. government's program to develop and acquire a safe smallpox vaccine based on the Modified Vaccinia Ankara (MVA) virus. "The Wave Bioreactor is actually easier to use when compared to a standard bioreactor," says Rene Djurup, chief technical officer of Bavarian Nordic. "With single-use containers, cleaning validation is not necessary, which saves valuable time," adds Karen Wassard, production director for Bavarian Nordic. "It was plug-and-play from about the first day," she continues.

Working together

Collaboration is another strategy that vendors have employed to take advantage of the burgeoning disposable-bioreactor technology market. Last October, Wave Biotech entered into a collaboration with vaccine innovator Novavax (www.novavax.com) on a commercial-scale process for the company's vaccine for the avian influenza virus, as well as other biological products.

In December, Fisher Biosciences (www.fisherscientific.com) teamed up with Alfa Laval (www.alfalaval.com) to provide

SHAKING UP BIOREACTOR TECHNOLOGY

A unique disposable bioreactor concept based on shake-mixing has been developed by researchers at Aachen Univ (www.biovt.rwth-aachen.de) and Hoffmann-La Roche Inc. (Roche; www.roche.com). The reactor assembly comprises 20-L or 50-L cylindrical vessels made of polypropylene or from transparent polycarbonate mounted on a standard shaking machine from Kühner AG (Switzerland; www.kuhner.com) with a maximum loading capacity of 200 kg and shaking frequencies from 20 rev/min to 400 rev/min.

"The mixing performance of disposable shaking reactors, 20-L and 50-L vessels for shaking frequencies larger than 80 rev/min, is very favorable," notes Chao Min-Liu, research leader of cell culture at Roche Discovery Technologies. "Mixing occurs only a few seconds after the addition of a tracer. Further, there is a well-defined gas-liquid mass transfer area, low levels of hydromechanical stress due to homogeneous distribution of power consumption, and sufficient oxygen transfer for growth of plant and animal cells," he continues.

Liu and his fellow researchers were the first to scale-up production processes based on the animal and insect cell lines in disposable shaking bioreactors from 3 L to 50 L. "In all the cases, cell growth was better than that obtained by spinner flasks or a standard fermentor," says Liu. Chinese hamster ovary (CHO) cells were grown in a fed-batch mode in a 50-L shaking bioreactor with a maximum viable cell count of 6×10^6 cells/mL. Despite their successful cultivation of different cell cultures, the researchers stressed the need for further characterization of these simple and efficient shaking bioreactors. "Our findings show that shaking technology is not limited to the milliliter scale. The maximum reactor size was 50 L, although the maximum permissible size of disposable shaking bioreactors 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, or space availability," notes Liu.

single-use bioprocessing systems to Haemacure (www.haemacure.com), a developer of bio-adhesives and biomaterials. The disposable systems will be used in Haemacure's new Hemasee fibrin bio-adhesives manufacturing facility. The bioprocessing systems are marketed and sold by Alfa Laval under an exclusive agreement with HyNetics (www.hynetics.com), a joint venture between Alfa Laval and HyClone (www.hyclone.com), a supplier of cell cultures and bioprocessing systems. The contract with Haemacure marks one of the first major installations of HyNetics technology for large-scale bioprocessing. HyNetic is launching a 10,000-L disposable bioprocessing unit this spring.

Contract manufacturers, such as PacificGMP (www.pacficgmp.com), are especially keen on disposables because they enable them to work more efficiently and practically eliminate contamination issues. PacificGMP's San Diego plant uses disposable technology to produce biologics. "We have chosen this type of processing over 'traditional' modes of production for the shorter time to market, which is crucial for smaller pharmaceutical and biotech companies. The capital investment is reduced and the cost of goods sold is substantially lower, says Leigh Pierce of PacificGMP.

Meanwhile, vendors are vying for market share by offering a greater diversity of design options (*e.g.,* larger-size bags featuring integrated tubing, filters, connectors, sensors), as well as standardized bag designs to meet the industry's demands for reliable, cost-effective and scalable performance.

"Because bioprocesses often require extensive use of sophisticated equipment, novel technologies that translate into faster and simpler operations, while reducing the impact on resources, appear very attractive," says Dream. At the basic level, the primary means of large-scale production of therapeutic proteins in the pharmaceutical industry is through batch production of cells or organisms that secrete the protein of interest. The primary means of large-scale production of therapeutic proteins in the pharmaceutical industry is through the use of bioreactors containing cells that have been modified to secrete the protein of interest.

The cell lines of choice include a tumor cell line, spleen cell/carcinoma combination, or a genetically engineered animal cell line called a hybridoma. These cells, since they are derived from mammals, can perform post-translational modifications such as attaching sugar molecules to the protein surface. They usually have desirable properties, like fast growth and immortalization, that bacteria and yeast have.

The Wave Bioreactor has been used for cultivation of suspension and anchorage-dependent mammalian cells, as well as insect cells for clinical production of activated autologous T-cells used in the treatment of various forms of cancers. A Wave-style apparatus consists of a sterile disposable plastic bag that is half filled with a cultivation medium, and the head space is filled with the desired gas mixture. The bag is placed on a rocking platform that delivers a wave-like motion to the liquid, thereby delivering adequate mixing and gas transfer to the culture, while preventing formation of damaging gas bubbles. By fitting the Wave Bioreactor with a floating filter, the system can be used as a perfusion reactor (where the cell bleed rate is varied to achieve an optimal cell density and specific growth rate), supporting cell concentrations of up to 3×10^7 cells/mL, a six-fold increase over concentrations routinely achieved in batch cultures.

Taking a different approach to perfusion is Integra Biosciences (www.integra-biosciences.com). "For bench-scale systems, our CELLine culture devices demonstrate perfusion mode using a compartmentalization approach that maximizes the cell density by separating cells with a molecular weight >10,000 from the nutrient medium via a semi-permeable membrane," says Fabrizio Baumann, product manager for Integra BioSciences. The concentrated cells are maintained in a small volume compartment. Hybridoma cells, lymphocytes and leukemic cells can reach concentrations that are 20–30-fold greater when compared to growth in static vessels.

Integration with convention

"The integration of single-use sensors and a pressure-control system with data logging and SCADA capabilities offers great opportunities for cGMP applications," says John Chorazyczewski, the president of Applikon (www.applikonbio.com). Capitalizing on this notion, Applikon is currently beta-testing eight CE-compliant AppliFlex units in the U.S., including one unit at Genentech, Inc. (www.genentech. com).

"Compared with traditional reactors, disposable bioreactors have the potential to increase the R&D pilot-plant capacity, while significantly decreasing installation costs and labor requirements," says Donna Giandomenico, an engineer for process development engineering at Genentech. Several studies were performed at scales from 1–400L in the bioreactor. "Results from large-scale monoclonal antibody production demonstrated comparable growth, titer and product quality to that of conventional stainless-steel bioreactors," says Giandomenico.

A complete plug-and-play system costs $28,000, while the disposable bioreactor bag alone costs $17,500. Applikon plans to incorporate technology that would measure and control viable cell mass, and simplify sampling methods for glucose and lactase.

As companies look for replacements of large-scale

fixed capital assets, biotech design firms are moving away from providing individual disposable components and toward whole systems that can replace traditional process equipment. Xcellerex, Inc. (www.xcellerex.com) is rolling out its XDR Disposable Bioreactor System — a self-sufficient skid-based unit operation. "The portable system integrates single-use, disposable product contact materials and optional localized environmental control in order to eliminate or minimize external utility systems and facility requirements," says Parrish Galliher, president of Xcellerex. The system is scalable from working volumes of 50 L to 2,000 L, and comes with a local control system that is adaptable to external data acquisition and distributed control systems. Batch automation is also available for controlling the sequence of process steps, capturing operator input and additional compliance supervision.

One of the main concerns with a new single-use system is ensuring that its operation and performance are similar to existing systems, especially with regard to oxygen transfer. HyClone's newly released Single Use Bioreactor (S.U.B.) is intended as a retrofit product to replace the stainless-steel bioreactor vessels in existing animal cell culture bioreactor systems, as opposed to installing a complete turnkey bioreactor system. "It consists of a reusable stainless-steel outer support container ($20,000–$25,000) and a bioprocess container (BPC; $2,000–$3,000) that integrates with an existing bioreactor control system," explains Jon Reid, business director at HyClone. One of its most noteworthy features is that it models a conventional overhead stirred tank, "so all of the traditional engineering scaleup calculations apply," says Reid.

"During tests, cell growth and antibody production in the S.U.B. were comparable with those in a conventional system," he notes. It is available in 50-L and 250-L maximum working volumes. A 1,000-L unit is in development for release in 2007. Reid says the firm is actively seeking partners with whom it can develop microbial fermentation systems and turnkey disposable bioreactors.

Caveats and limitations

Although disposable components are beginning to see more use in industry, the vast majority of bioprocess equipment in manufacturing facilities is fabricated from stainless steel. "Stainless steel is the material of choice due to its relatively high chemical resistance to most bioprocess buffers and the ability to electropolish it, which reduces surfaces that can harbor microbes and facilitates sanitary operations," says Geoffrey Hodge, vice president of technology for Xcellerex.

Disposable components have limitations. They may not be suitable for applications using either certain process buffers, or harsh chemicals and extreme temperatures. Plus, the extractables from the disposable components must be evaluated. The industry is addressing this issue by moving toward a common strategy for assessing extractables. "Many vendors can provide packages of extractables data for their materials, and a risk-based approach to assessing extractables has been proposed," says Hodge.

Vendors have also responded to the need for prevalidated equipment, which shifts the regulatory burden away from end-users. For instance, Applikon released an FDA-approved film for the single-use bags that are included with its recently unveiled AppliFlex Rocker. Made of Stedim71, a co-extruded six-layer film offering mechanical strength, high chemical resistance and low gas permeability, AppliFlex will initially be available in 10-L, 20-L and 50-L capacities, with 100-L and 200-L coming soon," says Chorazyczewski.

"Another consideration is that these systems cannot replace some unit operations, including many chromatography chemistries, and many of the small-scale components that are available may not be available or feasible for large-scale use," says Hodge. "Thus, any potential savings are offset by the increased cost-per-batch of the disposable components themselves, including waste removal. And in these cases, it is not immediately apparent how much cost savings disposables really provide."

Some studies have attempted to answer this question. One study, using a model constructed in collaboration with BioPharm Services (www.biopharmservices.com), extrapolated the probable cost of disposables for larger scales — 100 L, 1,000 L and 10,000 L — in cell-culture operations, downstream operations, and hold tanks for buffer, media and product. On the operating side, there is a 20% savings in utility costs and a 20–30% savings in cleaning and validation costs. There is also a 70% reduction in water usage (for cleaning), and thus 70% less discharge that must be treated for removal of acids, alkalis and cleaning agents. With such advantageous benefits, the future of disposables is bright.

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