

A Stirred-Tank Bioreactor

Delivered in Eight Weeks and One Hour

by Michael Fisher

The Challenge: It's time to scale up or add capacity. A critical drug is moving through process development or clinical trials.

In eight weeks it needs to be in your new production bioreactor, at scales ranging from 200 to 2000 L.

Can you specify, FAT (factory acceptance test), install, and start your bioreactor in that time frame? Yes, you can.

THE COMPONENTS

A fully functional bioreactor requires perfect integration of several components to ensure that cultures will finish at the desired productivity. These components include

- a jacketed stainless steel vessel
- temperature control
- agitation
- sparge gas control
- headsweep gas control
- pH and dissolved oxygen control
- a sampling mechanism
- weight indication and control
- cleaning hardware
- sterilization hardware
- piping and tubing to deliver all services, add media, pH control solutions, and gases
- feed and addition pumps, and
- a supervisory control and a data-acquisition system.

Once those components are specified, the facility layout needs to be designed to accommodate the reactor upon delivery, accounting for utility availability, footprint allowance, power, and accessory hardware.



XCELLEREX (WWW.XCELLEREX.COM)

Is all of that possible using a traditional, fixed stainless steel bioreactor? Most people will say that no, it is not. What makes it possible is application of disposable technology to bioreactor deployment. Here I examine each of the required components to see how a single-use disposable bioreactor allows the eight-week objective to be met and what additional advantages are provided in doing so.

Jacketed Stainless Steel Vessel: To provide a directly scalable operation for stirred-tank cell culture, a stainless steel tank will use established geometry in which working volume height is about 1.5 times the diameter. A tank with 30% volume for headspace and with sample points and probe installation points located at 20% of working volume allows the

system to operate at 5:1 turndown volumes and accommodate efficient strategies for headsweep action and foam management.

The tank will be insulated for efficient heat transfer. Using electric heating systems is possible, but they can be costly to operate and cumbersome to maintain or repair. A better option is to use a dimpled jacket surface to which is plumbed facility-chilled water or glycol. Additionally, for a completely independent bioreactor, incorporating a TCU (temperature control unit) to operate in closed or constant potable water feed mode will perform quite efficiently. An independent system promotes the ability to easily relocate the system.

Vessels designed for disposable bioreactors lack requirements for CIP

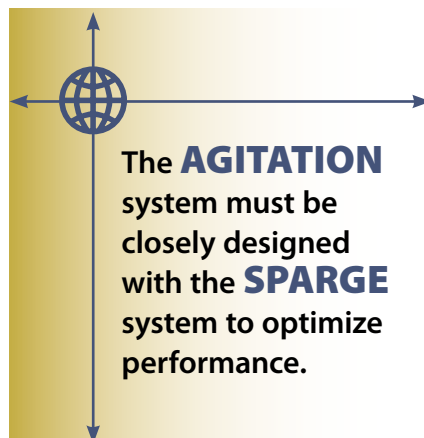
(clean-in-place) and SIP (steam-sterilize-in place) piping and ratings. In fact, an open-top tank is best and requires no ASME stamp for it or its jacket, because temperatures above 80 °C (polyethylene disposable film limit) are not needed. Open-top tanks provide plenty of light for seeing inside the reactor: A sight-glass placed longitudinally allows operators to visually monitor the culture. Sample ports and probe parts are part of the disposable assembly. The tank needs to provide access only to that portion of the disposable bag.

A jacketed stainless steel vessel of this design should be constructible from drawings in four to six weeks, and commissioning time is minimal because the tank's main purpose is to support a disposable assembly.

Temperature Control: Vessel temperature measurements must accurately represent the temperature profile. Any such measurement needs to be qualified with temperature mapping studies, for which the effects of agitation are determined. The advantage of disposable bioreactors is that temperature sensors do not need to contact process fluid. A simple thermocouple or surface resistance temperature detector (RTD) can be placed in the film or between it and the inner tank wall. Such installation points ease maintenance and calibration and eliminate concerns related to cleaning and sterilization. As an option, multiple temperature sensors can be installed for redundancy, profiling, or collecting additional data. Temperature measurements are fed back to a temperature control system.

Similarly, temperature exhaust filters are controlled to prevent condensation on filter elements. For a filter element, a small electric heating element will suffice, and an integrated thermocouple or RTD will provide sensing and feedback control. Typically, a filter element is held at 5–10 °C above the culture temperature.

Installation of sensors and controllers is simple and quick. Stand-alone TCUs for tanks of this size are available off the shelf typically in less than four weeks.



Agitation is a function that must be integrated into disposable assemblies. Several approaches are available including reciprocating bellows, rotating paddles, top-mounted shaft-driven propellers, and bottom-mounted magnetically driven impellers. Each agitation system needs to be evaluated for efficiency and measured as power-per-unit-volume to deliver oxygen mass-transfer rates required for higher-density cultures. The agitation system must be closely designed with the sparge system to optimize performance.

In all cases, the agitator is driven by an externally mounted drive. Additional features of agitation systems for disposable bioreactors include

- compliance of materials with USP Class VI and cytotoxicity standards
- penetration of components to the sterile envelope; shaft seals and insertions may not be desirable
- risk imposed during handling and installation of agitator assembly; although a 200-L bioreactor is about four feet tall, a 1000-L reactor is over six feet tall, and a 2000-L reactor is even taller
- risk imposed during operation of agitator assembly; moving parts need to be carefully performance tested, and
- safety of the system; operators need to be protected during system installation and operation.

Drive systems that use standard motors are commercially available in a matter of days.

Sparge Gas Control: The sparge element on a disposable bioreactor is integrated into the disposable assembly, with the boundary present at a hydrophobic filter. The system is completed by sparge gases fed to a

standard control system containing algorithms for gas control strategies and appropriate mass flow controllers, check valves, and tube delivery systems. Options for the sparge element include sintered stainless steel, ceramic, and polymer materials. In all cases, the element must be securely incorporated into disposable assemblies to ensure transfer of gasses through the pores. Often, multiple sparge strategies need to be used with different pore size elements and/or open tube gas addition. The disposable assembly must accommodate all of these strategies.

Headsweep Gas Control: The delivery of gas to a disposable bioreactor is integrated into the disposable assembly, with the boundary present at a hydrophobic filter. To complete the headsweep gas control system, headsweep gas is fed to a standard control system containing algorithms for gas control strategies and appropriate mass flow controllers, check valves, and tube delivery systems.

pH and Dissolved Oxygen Control: Dissolved oxygen and pH probes must be inserted into the sterile envelope of the bioreactor bag. The ability to insert more than one probe for redundancy, profiling, or collecting additional data is a significant consideration. Tank hardware should be designed to accommodate and support probe installation.

Standard electromechanical probes can be used for pH and DO₂ measurements. They are then integrated into a control system for gas-flow and pump control to ensure that a process value tracks setpoint. As fluorescent sensor technology advances, patches will be integrated into disposable assemblies, eliminating the need to add probes. Standard probes are available usually within one week.

Sampling Mechanism: Sampling for a disposable assembly is performed using disposable sample bags or syringe connections. No mechanical hardware is required.

Weight Indication and Control: Weight is a critical measurement for process control. Portable tanks can be



mounted on floor scales, and fixed tanks are typically installed on load cells. Integrating a weight indicator into a control system allows use of feeding by weight and alarming functions. Floor scales or load cells are commercially available and typically delivered in about four weeks.

Cleaning Hardware: No cleaning is required. All product contact surfaces are single-use disposable. At the end of each run, remove and discard the disposable components. Reload the system with a new set of disposables for your next run. This means that cleaning-related systems are also not required: tank, drains, water, cleaning skids, procedures, validation, and so on.

Sterilization Hardware: No sterilization-in-place is necessary. All product contact surfaces are prepared using gamma irradiation and arrive ready for installation and use.

Again, related systems are not required. Piping and tubing deliver all services, add media and pH control solutions, and gases. Simplified tubing systems are all that is required to deliver gases to the system. All remaining transfers are made through single-use disposable tubing.

Feed and Addition Pumps: Peristaltic pumps used to transfer fluids through tubing to the sterile envelope are required and should be integrated into the control system for optimum performance.

Supervisory Control and Data Acquisition System (SCADA): A strong SCADA system is an integral component to the performance of a bioreactor and should not be neglected. Few off-the-shelf packaged systems are available. Key features to consider are

- scalability (adding additional reactors to the system)
- batch management (electronic batch records)
- service and support capabilities
- validation approaches and quality of system development
- user operability (enabling users to configure control loops from the operator interface without the need for programming).

Bottom Line on All Hardware

Components: When specified with an eye toward full integration and an understanding of disposable assembly configuration, all components will be deliverable for integration within six weeks.

So far, so good.

THE DISPOSABLE ASSEMBLY

The disposable assembly is the critical piece of this reactor. Let's assume that materials selected meet your required quality and compliance attributes, and that filters, fittings, tubing and connectors are readily available.

Selecting a mixing strategy that makes sense is the next critical decision. This point is repeated here not only because the mixing device will be disposable, but because several key considerations factor into the selection.

Mixing designed for maximum gas transfer with minimum shear and vortexing is the objective. Make sure your selection is backed up by computational flow dynamics modeling and rigorous kLa (volumetric mass transfer coefficient) studies.

- Consider what will happen when scale increases to the maximum

available disposable assembly (currently 1000 L, probably 2000 L in the near future).

- How will the chosen configuration grow as scale increases?
- How will this affect storage, handling, and installation of the disposable assembly?
- How will this affect assembly cost?
- What hurdles will appear if you have to go to a stainless steel reactor at 5000 L, 10,000 L, or larger scales?

Once the agitation strategy is chosen, using the manufacturer's standard configuration for test batches allows quick startup and should provide a fully functional system. Using this strategy allows delivery of a disposable assembly within the eight-week objective. As your process matures, the disposable assembly can be customized to specific operating needs.

INSTALLATION

Now we have specified, built and tested a disposable bioreactor. Installation at the manufacturing site requires sufficient power and gas utilities and availability of growth media. Installation timelines, including startup and training, should take less than one week.

Key advantages once the disposable bioreactor is installed include

- batch-to-batch changeover in less than one hour
- mobility of the system within the plant
- greatly reduced utility costs (no water or chemicals for cleaning, no clean steam)
- increased reliability, and
- scalability.

Let's make 2006 the "Year of the Stirred-Tank Disposable Bioreactor": delivered in less than eight weeks and productive as near to 24 hours/day, seven days/week as possible. 🌐

Michael Fisher is vice president of operations and engineering at Xcellerex, where he is responsible for systems engineering, facilities, supply chain, and information technology; Xcellerex, Inc., 170 Locke Drive, Marlborough, MA 01752; 1-508-480-9235, ext 236; mfisher@xcellerex.com.

