

## Single-Use Technologies A Contract Biomanufacturer's Perspective

Disposable technology presents an attractive opportunity for CMOs to cut down production costs.

Imara Charles, Janet Lee, Yamuna Dasarathy



### Abstract

The closed and controlled environment of single-use technologies in a biopharmaceutical production process can significantly reduce processing time. A fully disposable option eliminates cross contamination, cleaning, and subsequent validation, reducing the use of water for injection. Lower operating costs, smaller equipment footprints, increased productivity, and rapid turnaround time are some of the major benefits that are driving the market demand for single-use technology in manufacturing. This article discusses a contract manufacturing organization's perspective on the use of disposable technology in the biopharmaceutical production process.

To eliminate contamination and to comply with the FDA cleaning regulations, manufacturers spend a lot of money, time, and resources on equipment cleaning and sterilization—steps that require large volumes of water for injection (WFI)—and cleaning validation. Most bioprocess equipment is made of stainless steel and requires assembly, clean in place (CIP), and steam-in-place (SIP), all of which result in manufacturing downtime. By using disposables, contract manufacturing organizations (CMOs) can save WFI costs by not having to clean the stainless steel bioreactors and validate the cleaning process every time.

These reasons have fostered the advent of single-use technology, which has been embraced by technologically inclined companies. These companies understand that significant investment capital is needed to construct a manufacturing facility. It costs \$10 million to construct, equip, and launch a 100-L traditional pilot plant facility. The cost goes up to \$40 million for a 1,000-L pilot plant and for a 10,000- to 20,000-L facility, the cost becomes prohibitive, and may be as high as several hundred million dollars.<sup>1,2</sup>

Because of their customizable design, disposables facilitate faster design, construction, and commissioning of facilities and offer manufacturing flexibility—a significant operational benefit. Reduced processing time, enhanced productivity, smaller and flexible production facility, and reduced operating costs are some of the major benefits that are driving the market demand for single-use technology.

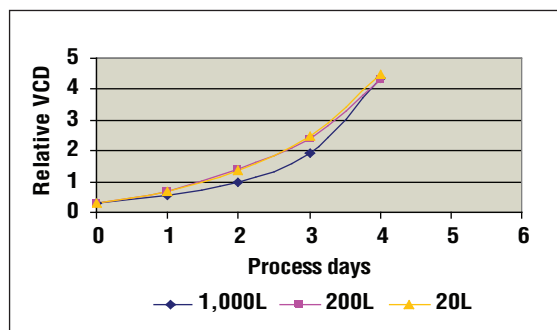
### Financial Considerations

Contract biomanufacturing companies are continually striving to improve and optimize the process of manufacturing biotherapeutics at every stage, starting from pre-clinical toxicology material to large-scale current good manufacturing practice (cGMP) commercial production. A contract manufacturer on average makes a 33% profit in manufacturing a biotherapeutic.<sup>3</sup> In order to be competitive and be commercially viable, CMOs have to make improvements in this area. They have a pressing need to economize the manufacturing process, skillfully manage their project pipeline, and help their sponsors accelerate their speed to clinic or market.

Process economics is not for just the dedicated CMOs, however. A biopharmaceutical company with inbuilt capacity is in a similar situation, spending more than 20–25% of its operational cost on manufacturing,<sup>4</sup> and is equally interested in lowering the production costs. Disposable technology presents an attractive opportunity to cut down production costs for both biopharmaceutical and contract manufacturers.

### Where Are Disposables Used in a Biomanufacturing Process?

Single-use filters, single-use tangential-flow filtration (TFF) membranes, flexible tubing, sterile liquid containment bags, disposable bioreactors, mixing systems for media preparation, sterile bags with normal-flow filter manifolds, and aseptic connectors are all used in appropriate steps during the process. These disposables optimize processes and accelerate biomanufacturing production time. In downstream processing, dis-



**Figure 1.** A comparison of growth rates in viable cell density (VCD) of Chinese hamster ovary cells in Wave bioreactors of different capacities—20 L, 200 L, and 1000 L.

posable formats are used for cell removal, harvest clarification, membrane absorption chromatography steps, viral filtration, ultrafiltration, and diafiltration. At Laureate, we use single-use technology at all of these steps throughout the biomanufacturing process.

### Cell Culture

For small-scale culture scale-up, we routinely use disposable flasks and roller bottles. For larger scales up to 1,000 L, we use disposable Wave cell bags.

### Bioreactors

Wave disposable systems range from 1 L to 500 L in capacity, and we use them in conjunction with disposable cell bag 20/50, 100/200, or 500/1000 Wave bioreactor systems. These are customized systems that use rocking motion to mimic the agitation produced by stand-alone systems. They have built-in feedback controls to monitor carbon dioxide, dissolved oxygen, pH, and other parameters. Our studies have shown that cell growth in these systems for most cell lines (Chinese hamster ovary [CHO] cells, BHK, hybridoma) is comparable to that obtained from stand-alone systems (data not shown). We observed similar growth trends throughout the run when we scaled up from a 20-L to a 1000-L wave bag (Figure 1).

Another type of disposable bioreactor uses hollow-fiber (HF) cartridges. In such perfusion

Imara Charles, PhD, is a senior scientist and Yamuna Dasarathy, PhD, is a senior manager, marketing, both at Laureate Pharma, Princeton, NJ, 609.919.3393, yamuna.dasarathy@laureatepharma.com. Janet Lee was an upstream processing specialist at the time of submission and has since left the company.

bioreactors, cells grow inside cartridges, around a semipermeable 10-kD hollow fiber membrane. Cells are fed and waste is removed by perfusion across the membrane. HF disposable bioreactors facilitate a plug-and-play approach to cell culture, and have the advantage of concentrating the product produced by the cells.

### Downstream Processing

Various vendors supply bioprocess containers (BPC) for solution preparation. These containers are made of polyethylene, ethylene vinyl alcohol (EVOH), and other films. They are available in a variety of sizes ranging from 25 mL up to 500 L, and we transport them in re-usable barrels or totes. BPC systems are completely configurable for various uses, including direct connection to chromatography systems or process tanks. The bioprocess containers are tested to United States Pharmacopeia (USP) Class 6 standards before use. The vendor performs the following tests and confirms validation at the time of purchase:

- acute systemic toxicity
- intracutaneous toxicity
- implantation test
- cytotoxicity (agar diffusion and elution)
- endotoxin level
- heavy metals concentration
- buffering capacity
- nonvolatile residue
- residue on ignition.

### Membrane Chromatography

Disposables are an integral part of membrane chromatography for protein purification. We use cation and anion exchange filters from various vendors to replace cation and anion exchange resins and columns. The cost analysis shows that disposable membrane filters are substantially more cost-effective than stand-alone columns and resins. In addition, by using these filters we have eliminated the need for packing and testing columns as well as long-term storage of materials.

Anion exchange filters are typically flow-through (FT) systems that bind rDNA, endotoxin, residual protein A, and host cell proteins, and we use them for polishing steps. A comparison study of Q filters from two different vendors (Figure 2) shows that disposable membrane filters generally result in >90% yield postprocessing. We have observed similar yields when we run process-scale columns packed with Q anion exchange resins.

Cation exchange filters act as product-binding filters similar to cation exchange resins and bind

IgG and Factor VIII. Cation exchange filters, however, have lower capacity than most resins and are not efficient for intermediate purification steps.

### Virus-Removal Filters

We use different types of disposable filters mentioned below for virus removal (Table 1).

#### Hollow Fiber Filters

In a disposable HF filter, the product is ideally filtered through the hollow fiber membrane by pressure at 14 psi. Total yields are greater than 95% and membranes are scalable to 4.0 m<sup>2</sup>. However, a pressure tank is necessary if pressure feeds are used and therefore, this system is not fully disposable in its ideal configuration. We have observed that this system can function at 120–150 L/H/m<sup>2</sup> and can achieve greater than 6.7 log removal of polio virus (vendor study).

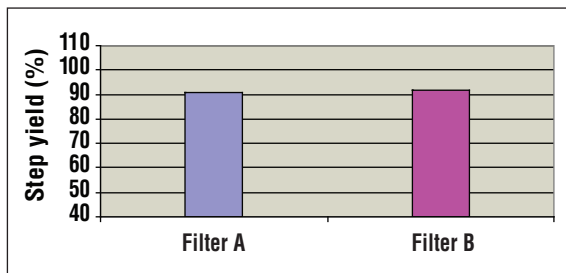
#### TFF Membrane Filters

TFF membrane filter cartridges are composed of three disposable membrane modules connected in parallel. However, stainless steel membrane holders are needed for setup and therefore, this system is not fully disposable as it has nondisposable parts. The TFF system functions at 50–80 L/H/m<sup>2</sup> and achieves greater than 6.0 log removal of retrovirus (vendor study).

#### Cartridge Filters

Polyvinylidene difluoride (PVDF) filters are completely disposable cartridge filters that can be hooked up directly to pumps or chromatography systems. The product is pumped through the filter in a single or parallel channel configuration. The filters are scalable to 30 inches and can be connected in tandem to polishing columns, if needed. We have observed that these filters function at flow rates ranging from 5–20 L/H/m<sup>2</sup>. The PVDF filters are commonly used in the industry and have successfully removed greater than 6 logs of retrovirus in our processes and in other commercial applications.

**Figure 2.** A comparison study of Q filters from two different vendors, used for the polishing step of a monoclonal antibody purification process. Three process yields were averaged for each filter.



However, backpressure issues are common, resulting in slower and less efficient virus removal steps.

Polyether sulfone (PES) cartridge filters are also available for viral removal steps. PES filters are hooked up to pumps or chromatography systems in the same fashion as PVDF. Scalable to 30", these filters are completely disposable and can be hooked in tandem to polishing columns, if applicable. Our studies confirm that PES filter flow rates are higher than PVDF filters at 90–180 L/H/m<sup>2</sup>. We performed a virus validation study using PES filters by pumping virus-spiked product through small-scale cartridge membranes at >30 L/m<sup>2</sup> for all runs. Results show that the product spiked separately with either 1% or 5% (v/v) of four viruses (Reo, MVM, MuLV, or PRV) behaved in a manner consistent with our scaled-down processes. In all cases, the flow-rate curves were linear, suggesting that the filters never exceeded the virus binding capacities. Studies also confirmed greater than 6 log reduction in all of the above named viruses.

### Aseptic Filling

For aseptic filling of liquid parenteral products we use completely disposable sterile bags as bulk product containers and have seen excellent recovery. One caveat to using disposable bags for aseptic filling is that the users must confirm that the product solution is fully compatible with the plastic material of the bag. Our linear peristaltic pump systems have completely disposable parts, including

hoses and filling needles to accommodate vials from 0.2 to 100 mL in size with fill volumes of 0.1 to 100 mL (Figure 3).

### A Case Study

To demonstrate the feasibility of using single-use technology at various steps throughout the biomanufacturing process, we cite below a case study on the usage of disposable bioreactors.

**Table 1.** A comparison of four types of filters commonly used in a post-chromatography purification process for virus removal. Flux rates, recovery, and convenience of use were studied for each filter for different processes.

Filter type	Virus removal claims	Flow rates (L/h/m <sup>2</sup> )	Recovery	Fully disposable?
Tangential flow filtration module	> 4 log bacteriophage > 6 log retrovirus	50–80	85–90	N
Hollow fiber	> 6.2 log parvovirus > 6.7 log polio virus	120–150	95–98	N
Polyvinylidene difluoride	> 3 log bacteriophage > 6 log retrovirus	5–20	80–90	Y
Polyether sulfone	> 4 log bacteriophage > 6 log retrovirus	90–180	90–95	Y

**Figure 3.** Filling machine with disposable parts



### The Challenge

A biotechnology company developing monoclonal antibody-based therapies, approached us to produce preclinical material for toxicology studies and cGMP material for Phase 1 studies. The client company had a well-developed process that was ready for technology transfer. The company had completed cell line and media development and was successful in scaling up to small-scale (5 L) bench-top bioreactors. However, they had no capability for large-scale production. The client wanted us to complete technology transfer and generate toxicology materials within six months, followed by production of Phase 1 materials within nine months.

### The Solution

The amount of cGMP therapeutic material required for Phase 1 stipulated that we use a 2000-L stirred-tank bioreactor. We developed a successful process flow starting with a vial of master cell bank cells and scaled them up in sequence in Wave 20-L, 50-L, 200-L and 1000-L bioreactors to inoculate our 2000-L stirred-tank bioreactor for production.

One of our challenges in the process was to scale up the high density and high antibody

yielding CHO cells in the 1000-L Wave bioreactor to generate inoculum for the 2000-L stirred-tank bioreactor. To sustain the maximum viability of the cells in this large vessel, we achieved the optimal mixing speed by adjusting both the angle of rocking and the rock rate. Using this model, we prepared the entire seed train using Wave disposable bioreactors and generated inoculum for the 2000-L production reactor.<sup>5</sup> The scale-up was successful and the process yielded the desired product volume for the Phase 1 clinical study. By using single-use technologies, we were able to eliminate time- and resource-consuming CIP procedures and streamline the entire manufacturing process. We completed the optimized scale-up process within the required timeframe and budget.

### The Roadblocks

One of the challenges of adopting single-use technology is that not all cell lines are compatible with disposable bioreactors. We have observed that an antibody-producing NSO cell line did not grow in Wave bioreactors in the

**One of the challenges of adopting single-use technology is that not all cell lines are compatible with disposable bioreactors.**

presence of chemically defined serum-free growth media with cholesterol. NSO cells require that external cholesterol be added to the media as a supplement. However, we found that the cells grew poorly and adhered to the surface of the bag. This phenomenon was presumably because of the cholesterol, as the problem could be overcome by selecting NSO cells that did not require cholesterol.<sup>5</sup>

The second challenge in implementing dis-

posable technologies is that the biggest disposable bioreactor commercially available in the market is only 1,000 L in size. If a process needs to be scaled up to a higher volume, then the process must be transferred to a stainless-steel bioreactor (as cited in the above case study).

Adopting single-use technology poses a major challenge in downstream processing and it needs to be developed further. Ion-exchange filters do not have the same capacity as ion exchange resins, and are therefore of limited value in product-binding steps. This is a significant limitation for scaling up ion exchange or affinity filters for large-scale chromatography steps. Although single-use technology has come a long way, it has to satisfy the needs of downstream processing before a completely disposable biomanufacturing process can be developed.

### Acknowledgements

We would like to thank Michiel E. Ultee, PhD, senior director of process sciences, for his critical review of the manuscript. **BP**

### References

1. Schultz TJ. Validation of an emerging trend in vaccine manufacturing: disposable technology, filters, containers, and connectors. Disposable technologies for biopharmaceuticals. Institute of Validation Technology; 2007 Feb 27–Mar 21; Alexandria, VA.
2. Mintz CS. Developing and managing successful bio-outsourcing relationships. D&MD Publications; 2006 Mar;3:Chapter 3–1.
3. High Tech Business Decisions. Biopharmaceutical contract manufacturing—quality, capacities, and emerging technologies. 2007; Chapter 5–71.
4. Waele KD, Vandermarliere M, Broekhoven AV, Vandiest R, and Pendlebury D. A novel single-use mixing system for buffer preparation. Bioprocess Int. 2007 May (5) Supp 4:56–61.
5. Lee, J. Case studies of culture scale-up of production cells grown in disposable-bag bioreactors. A presentation at IBC's 2nd international biopharmaceutical manufacturing & development summit, Dec 6–7, 2006; Orlando, FL.