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COVER STORY

Disposable Plastic Bioreactors Lead To Savings—And Challenges—For Biopharma Firms

Single-use bags and other products are making inroads in protein drug manufacture, but the compounds they leach spell trouble

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ROCKING OUT
Plastic bioreactors come in many shapes and sizes. Here, a researcher uses a square bag in a rocking mixer.
Credit: GE Healthcare

Picture the process that biopharmaceutical firms currently use to manufacture protein drugs. You probably imagined scientists growing large numbers of animal or plant cells in huge stainless steel vats. The cells churn out designer proteins such as monoclonal antibodies, and the scientists “harvest” and store those biologics for treating patients with conditions such as rheumatoid arthritis.

The giant vats are certainly still a fixture in biopharma manufacturing facilities. But there’s a new cell culture system in town: Over the past decade, facilities have begun using enormous disposable plastic bags.

That may sound odd, but the single-use plastic systems, as they’re called, have a lot going for them. Because they’re sterile and tightly sealed, they cut down on the risk of microbial contamination between drug batches and during cell growth. They also eliminate the need for manufacturers to clean and sterilize vats between batches—an expensive process that requires lots of time and water.

The bioreactor bags now commonly come in volumes of hundreds of liters and range up to 2,500 L, says James D. Vogel, director of the **BioProcess Institute**. “You can buy them right now, commercially ready to go,” Vogel says. “It costs up to thousands of dollars to buy the bag. But if you think about what it costs to steam a tank, clean a tank, and all the validation, people have justified them.”

According to the most recent survey of biopharmaceutical manufacturing and production companies by marketing research firm **BioPlan Associates**, more than 90% of respondents use some sort of single-use component, with more than 70% of respondents using single-use bioreactor bags.

But single-use systems aren’t without their challenges. From a materials science standpoint, the biggest of these challenges is dealing with so-called extractables and leachables—chemicals that migrate out of the plastic and, in the case of bioreactors, into the cell culture inside the bag. Another challenge is the lack of standardization among suppliers of disposable systems and components.

Today, plastic disposables—including tubing, storage containers, and bioreactors—are available for all the steps of upstream and downstream biopharma processing. “The first complete single-use production facilities are in operation or under construction” for making biologics and their generic counterparts, biosimilars, says **Regine Eibl**, a professor at the Institute of Biotechnology at Zurich University of Applied Sciences. She points to facilities run by WuXi PharmaTech, an R&D service company in Shanghai; Alvotech, a biosimilars manufacturer in Reykjavik, Iceland; and Polpharma Biologics, a biosimilars and manufacturing service company in Gdansk, Poland. In addition, Amgen opened a single-use manufacturing plant in Singapore last year.

Even with all the advantages of single-use systems, the biggest driver to adopt them is that they enable firms to ramp up biopharmaceutical production capacity quickly with a small up-front capital investment, says **Thomas C. Ransohoff**, vice president and principal consultant at BioProcess Technology Consultants.

So far, companies have mainly been adopting single-use technologies for manufacturing biologics or biosimilars that will be administered in clinical trials, Ransohoff says. That’s a risky period because it’s hard to predict whether the product will pan out. “Any technologies that can reduce investment during that high-risk period or can increase the ability to move products forward have increased value for our industry,” he adds. If products manufactured with these technologies pass the testing stage and reach the market, the use of disposable systems for commercial manufacturing will no doubt increase.

Models constructed by Ransohoff's organization estimate that a typical monoclonal antibody manufactured in a 2,000-L single-use facility costs 25–30% less than one manufactured in a stainless steel facility at the same volume. At volumes beyond those achievable with single-use systems, economies of scale can make the stainless steel system preferable, Ransohoff says.

"There's a debate in industry as to whether conventional large-scale stainless steel facilities are more cost-effective than single-use facilities," Ransohoff says. "Until there are more data on a large commercial scale, I think the verdict's still out."

A typical single-use bioreactor is a plastic bag made of a multilayered polymer film. The three main layers are the inside layer, which is in contact with the cell culture; a barrier layer, which prevents gas diffusion into and out of the bag; and an outer layer, which provides mechanical stability. Those layers are "glued" together with tie layers that bridge their disparate chemical properties. "These films are pretty high quality," Vogel says. "We're not taking a Glad bag and putting culture in it."

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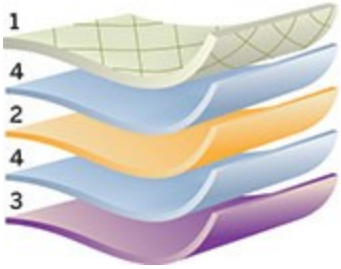

Layer	Thickness	Typical materials	Function
1 Outer	20–150 µm	Polyethylene, nylon, polyesters, polyamides	Abrasion & puncture resistance, strength, feel
2 Barrier	5–50 µm	Ethylene vinyl alcohol	Prevent transmission of O_2 and CO_2
3 Product contact	50–300 µm	Various densities of polyethylene	Biocompatibility, strength, sealing
4 Tie	Various	Modified polyethylene	Bonding between layers

PEELING BACK THE LAYERS
Single-use bioreactors are made of polymer films with multiple layers that serve different functions. The most common polymer in the films is polyethylene.
SOURCES: GE Global Research and other companies

To make these single-use bags and storage containers, manufacturers get their raw materials from chemical companies such as Saudi Basic Industries Corp. (SABIC). Amy Plançon, a marketing engineer at SABIC, says her firm is getting used to the new single-use market: "As our understanding of the single-use industry increases, we are learning that certain additives used in the production of polyolefins may not be suitable for use in single-use systems."

GE Global Research, which works with GE Healthcare to develop single-use products, chooses its polymers based on the mechanical properties and the desired look and feel for a particular application, says Andrew Burns, a materials scientist at the company. "Then we choose additives based on trying to make a film out of those materials,"

Burns says. The additives help the firm tweak the viscosity of the polymers so that the plastics can flow into a mold to make films.

But for the first generation of single-use films that companies manufactured, they repurposed existing technology used in IV bags or other medical applications, Burns says. “It let single-use get off the ground without having to invest dramatically in film design,” he says. “Now the industry is starting to take a new look at films.”

In making the next generation of films, “both mechanical and chemical properties in the entire bag need to be well-balanced,” Plançon says. “These bags need to withstand significant stresses throughout their life cycle, and the correct polymer materials need to be chosen in collaboration with stakeholders in the value chain.”

By far the most common material used to make the bioreactor bags is polyethylene, in all its various forms. “Polyethylene is a well-understood polymer,” says **J. Christopher Love**, a chemical engineering professor at Massachusetts Institute of Technology. “It can be made in many different densities. It’s cheap to manufacture and easy to work with.”

To process polyethylene into single-use products, however, manufacturers typically add antioxidants to help protect the polymer during the extrusion process used to make it into a film.

“Ultimately, if a polymer producer doesn’t stabilize the material, there is the risk that the single-use system won’t do its job properly,” says Marnik Vaes, a senior technical marketing manager at SABIC. “Film quality can be impacted, bags can rupture, and extractables and leachables can increase.”

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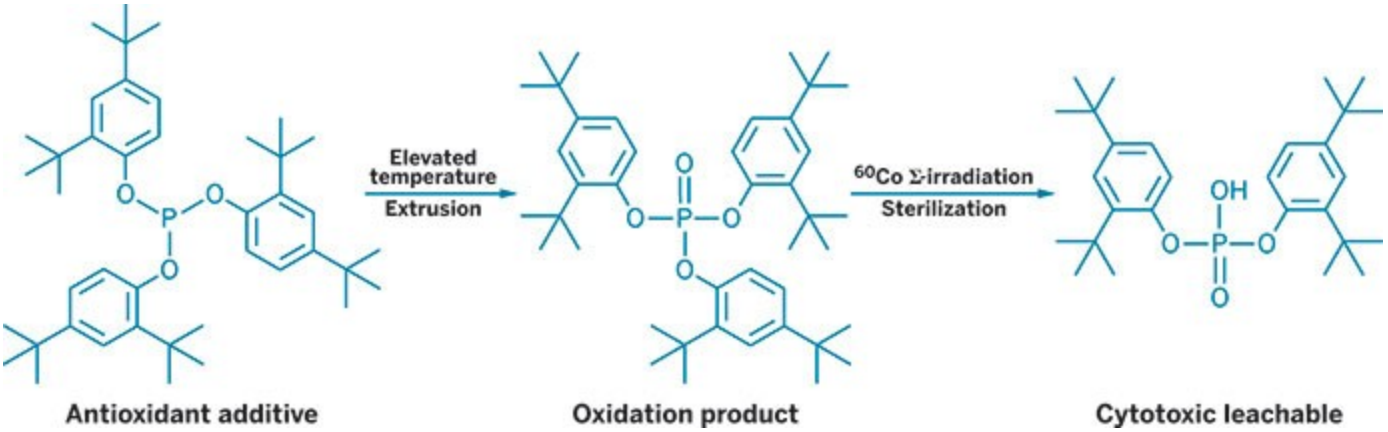

BAD ACTOR

The antioxidant additive *tris*(2,4-di-*tert*-butylphenyl)phosphite breaks down as a result of extrusion and sterilization to form the cytotoxic leachable *bis*(2,4-di-*tert*-butylphenyl)phosphate.

Those extractables and leachables are one of the biggest concerns about disposable bioreactors among biopharma firms today. The two classes of compounds are defined by the conditions under which they migrate out of the plastic. Extractables are pulled out by harsh solvents, high temperatures, or long reaction times under exaggerated conditions. They typically represent the worst-case scenario for how a product might be treated. In contrast, leachables migrate out of the plastic under normal operating conditions. In both cases, the compounds are either part of the plastic formulation or are by-products that form when a single-use product is made or sterilized.

Migration of compounds through materials can be predicted with modeling tools originally developed for food packaging, says Olivier Vitrac, a researcher at the French National Institute for Agricultural Research. Such modeling "cannot be used to predict breakdown products, but it can be used to predict their migration or the maximum amount that could be acceptable in the materials," he says. The results can then be used to make decisions about redesigning or reformulating materials.

One extractable—*bis*(2,4-di-*tert*-butylphenyl)phosphate (bDtBPP)—has a particularly bad reputation now. bDtBPP is a degradation product of the antioxidant additive *tris*(2,4-di-*tert*-butylphenyl)phosphite (TBPP). It typically forms as a result of a combination of the extrusion and sterilization processes, says Isabelle Uettwiller, head of the validation lab at Sartorius Stedim Biotech.

During extrusion, heat increases the formation of an intermediate degradation product, Uettwiller says. This intermediate then becomes bDtBPP during sterilization, which manufacturers carry out with gamma radiation.

Scientists at Amgen found that bDtBPP adversely affects cell cultures. When Matthew Hammond and coworkers spiked 0.8-mg/L bDtBPP—an amount they typically saw in film extracts—directly into Chinese hamster ovary cell cultures, nearly all the cells died (*PDA J. Pharm. Sci. Technol.* 2013, DOI: **10.5731/pdajpst.2013.00905**). In other experiments, Hammond and coworkers cultured the same kind of cells in a medium that had been stored in single-use bags for several days at 37 °C. In that case, cells grew more slowly in medium from bags shown to leach bDtBPP (*Biotechnol. Prog.* 2014, DOI: **10.1002/btpr.1869**).

Those cell lines were particularly sensitive, but similar issues have been seen with various cell lines across the industry, says Ekta Mahajan, a senior process development engineer at Genentech. Some people suggested removing the parent antioxidant from polymer formulations, she says.

But the answer to the problem was not removing the antioxidant. It was pinpointing how much of the antioxidant was needed, Mahajan says.

For example, GE reduced the amount of TBPP in its films to a point where the resulting bDtBPP concentration wasn't harmful to cells, says Burns, a materials scientist at the firm. "If you go to a completely antioxidant-free environment, you can end up compromising the properties of the film. We tried to find the right balance."

Similarly, Sartorius has developed a film that produces a minimal amount of bDtBPP. The company is also optimizing extrusion conditions to minimize production of the intermediate that leads to bDtBPP, Sartorius's Uettwiller says.

In addition to slowing cell growth, single-use containers can affect protein drugs stored in them. For example, monoclonal antibodies stored in plastic containers can form protein aggregates that decrease biopharmaceutical performance, Nina Xiao, a researcher at Genentech, reported last month at an **Engineering Conferences International** **meeting on single-use technologies**. She and her colleagues stored monoclonal antibodies for 12 weeks at 25 °C and characterized the resulting aggregates by size-exclusion chromatography. She attributed the aggregation to compounds that leached from the storage container.

Preheating bags before storage reduced the aggregation but didn't eliminate all of the leachables. Xiao could block some of the aggregation by adding tryptophan to the antibodies, which suggests that oxidation contributes to the aggregation, she said. In addition, adding the surfactant polysorbate 20 also reduced aggregation.

As companies work to develop new films, they are constrained by the inherent conservatism of the highly regulated biopharma industry. "This is not a space where we can release a new film product every year. We need to do a good job of engineering them the first time because we're going to have them for quite a while," Burns says.

"We have to try to futureproof as much as we can," Burns continues. "The biggest challenge is anticipating what's coming down the road and designing to that while at the same time not so overdesigning that it's way ahead of its time."

One way to improve everybody's understanding of the challenges involved with single-use systems is to get the major players—people from academia, biopharma, equipment vendors, and suppliers further up the supply chain—to talk to each other. For that reason, Mahajan organized the **Engineering Conferences International's** meeting on single-use technologies held last month.

"We wanted suppliers to understand our constraints from the end-user perspective," she says. "Why are we asking for these things? It's difficult for them to understand why the requirements are changing. At the same time, the end users need to understand the science of what goes into film extrusion and component manufacturing. What could be the limitations? Vendors might not always be able to meet what we're asking for."

Such conversations are increasingly important because there's currently a push under way for standardization of single-use products. In some cases, standardization is a matter of getting the same kinds of information from all suppliers so all users can easily compare products from different vendors.

Some of the earliest efforts in that area relate to extractables and leachables testing. "Right now we get data from suppliers in totally different formats, different solvents, and it's challenging and time-consuming to try to qualify," Mahajan says. "The idea is to simplify the process and get the basic information from them so we can focus more on the application and implementation instead of spending a year testing their system."

Mahajan has been involved with the push for extractables and leachables standards from the beginning. Those efforts have led to a drive for other standards too.

Standards are needed because drug manufacturers have in a sense outsourced a large part of their quality control to vendors and the companies that supply raw materials for single-use systems.

"With respect to qualification of raw materials, manufacturers are working with vendors to look further upstream in their supply chain than they did previously," Love says. "If you bought polyethylene, maybe you didn't care where that polyethylene came from. But different polyethylene lots from different suppliers may be different. You want to

understand that. Testing of raw materials is something that typically manufacturers would do themselves because it's a source of risk in their manufacturing process. But now biopharma companies are engaging their suppliers to share more of that burden."

The efforts "are being well-received by suppliers because it is to their benefit as well," Mahajan says. "We've been working one-on-one with suppliers to bring them up to GMP [Good Manufacturing Practice] requirements and explain why those are critical. When we're working with stainless steel, we have control over the quality." Now, with single-use plastics, biopharma is relying on suppliers to help with quality control.

The uptake of single-use technologies has been more widespread and rapid than people originally anticipated, says Ransohoff of BioProcess Technology Consultants. "It's exciting to see how enabling it is for companies that want to bring capacity on quickly, whether they're making biosimilars or new products," he says. "It won't replace conventional facilities completely, but it's certainly expanding the tools that we have to address challenging processes."

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