AN INNOVATIVE APPROACH TO MANUFACTURING BIOLOGICS USING MICROBIAL FERMENTATION

INTRODUCTION

Twenty years ago biologics were just beginning to emerge. Today, the global market for biologics has grown to almost a quarter the size of that for small-molecule drugs. In 2017 alone, 12 new biologics were approved in the U.S., 10 in the European Union, and 7 in Japan. "The opportunity to develop protein therapeutics that are intrinsically safe and potentially efficacious for a wide range of diseases makes them a very attractive class of drugs when compared to small molecules," explains J. Christopher Love, a professor of chemical engineering at the Massachusetts Institute of Technology (MIT).

Biologics, also known as biopharmaceuticals and biological drugs, are manufactured using living cells or microorganisms. They are large, complex molecules that are inherently more difficult—and therefore costly—to manufacture than their small-molecule counterparts. The global biologics market is expected to continue its upward trajectory. The 5.4% annual market growth rate seen in 2017 is expected to reach 9.6% in 2021, according to a recent report by the market research firm The Business Research Company. This expansion will be largely driven by the more than 1,000 new biologics under development, which are a mixture of innovative drugs, biosimilars, and biobetters.[1]

But as the popularity and numbers of biologics have grown, so has the engineering know-how on how best to manufacture them. "The opportunity to think about biologics for a variety of different diseases, and the handle that we now have on developing those from an engineering perspective, has catalyzed a lot of the activities and emerging innovation in manufacturing," Love says.

GROWTH IN MICROBIAL FERMENTATION MARKET

In 1982, humulin, a recombinant human insulin grown in the bacterium *Escherichia coli*, became the first biologic to be approved by the U.S. Food and Drug Administration. Eight more biologics were approved in the 1980s: four manufactured in *E. coli*, one in yeast, and three in mammalian cells.[2] Mammalian cell fermentation quickly overtook microbial fermentation as the main production method for biologics, and it has dominated the market ever since.

In recent years, however, microbial fermentation has been fighting back. Biotechnology companies are primarily attracted to reduced fermentation times and related lower costs. "The microbial side is projected to grow even faster than the mammalian side in the coming years," states Eric S. Langer, president of the biotechnology, pharmaceutical and healthcare publishing and market information service provider BioPlan Associates in a 2016 ***Genetic Engineering & Biotechnology News*** article.[3] "New microbial technologies will be part of the ecosystem that is pushing the growth in microbially produced biologics," he adds.

Susan Dexter, managing director of Latham BioPharm Group, a life sciences consultancy based in Cambridge, Mass., agrees. "Mammalian cell fermentation will be in the picture for quite some time, but microbial is becoming more and more asked for," she says.

BioVectra's Biotech Process Development lab provides biotech scale up and scale down process development capabilities.

Mammalian cell fermentation dominates the market due to the cells' ability to fold complex proteins, such as antibodies, correctly. Microbial fermentation requires a refolding step for complex proteins, which historically was technically challenging. "The technology for microbial fermentation has advanced to allow more complex proteins to be produced in yeast and ***E. coli***, where previously a mammalian cell culture would have been needed," says Jim Stout, director of process science biologics at the Canadian contract development and manufacturing organization (CDMO) BioVectra.

The new wave of biologics is also playing a part in the microbial fermentation boom. As researchers better understand what part of a molecular structure is required to get the desired efficacy, it becomes possible to make smaller molecules, Dexter explains. These smaller fragments, often referred to as third-generation biologics, are particularly well suited to microbial fermentation.

PLATFORM TECHNOLOGIES

Drug development, be it biologics or small molecule, is always a race against the clock. Getting to market quicker equals more sales before another innovative drug targeting the same disease gets approved or a patent expires, allowing biosimilars or generics to be launched. A manufacturing platform is an increasingly popular way to speed things up.

"A platform is a process of proven steps in sequence that a vendor would plug a client's product into and just optimize the parameters that are necessary on a molecule-by-molecule basis," Dexter explains. The manufacturer then optimizes each predetermined process step; the manufacturer doesn't lose time considering which process steps are needed. "You only go outside the platform if the platform is not providing the drug product quality or yields that you need," Dexter adds.

BioVectra's Biologics Process Development Suites

"With the platform technology, there is a base of knowledge that arises from utilizing that technology. It makes each new project slightly simpler because of the experience gained with previous projects," explains Heidi Hoffmann, senior director of manufacturing at Sutro Biopharma in South San Francisco, California. Sutro Biopharma uses a platform for the cell-free protein synthesis of its novel antibody-based therapeutics. A platform makes drug development "much more streamlined," Hoffmann says.

BioVectra will use a platform for biologic manufacture using microbial fermentation at its Windsor, Nova Scotia, site. The first—optional—step in this platform is a proprietary library of cell lines and a proprietary vectoring technology, developed by industrial partners. "Small companies don't usually have access to expression technology for making a microbial host cell system that will express the protein," Dexter says. One advantage of going to a CDMO that has that capability in-house is that you can start the process development cycle before the master cell bank has been finalized. This can shave up to five months off your timeline.

Automated high-throughput systems speed up optimization of each platform step. "They offer the ability to do many experiments in parallel," Hoffmann says. High-throughput fermentors enable the optimum upstream fermentation conditions, such as pH, dissolved oxygen, agitation, and temperature, for a candidate molecule to be rapidly determined.[4] High-throughput systems can also be used for downstream purification steps. The cleanup of a fermentation broth can be rapidly tried using numerous column-packing materials and conditions.[5]

"It's very quick to screen different chromatography parameters," Hoffmann says. BioVectra will use high-throughput systems, such as Ambr and Tecan, to optimize both upstream and downstream stages.

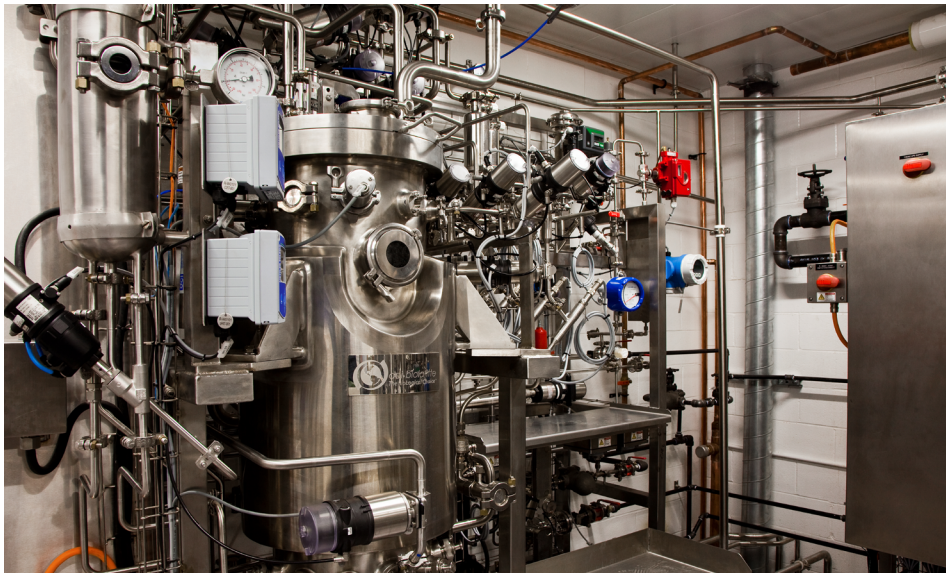
SINGLE-USE MANUFACTURING

Single-use disposable technologies are seeing increasing use in biologics manufacture, thanks to attributes such as improved operational efficiency, reduced operating costs, improved consistency, and minimized contamination risk.[6,7]

"There are a number of benefits to single-use technologies, the least of which is it speeds up turnover in the facility. You don't have to do all the constant cleaning on reusable equipment," Dexter says. This is a huge bonus for fermentation manufacturing, where cleaning is notoriously laborious, and it saves time and water, both expensive commodities for biotech facilities.

Sutro Biopharma uses entirely single-use technology for its cell-free reactions. "We used disposable technology so that we could turn over the suite for different products very efficiently," Hoffmann says. "We also don't have to figure out how to clean anything or how to prove that it's clean."

Preventing cross-contamination from one product to the next is a must in biologics fermentation, so extensive cleaning is needed for traditional stainless steel fermentors and downstream tools before they can be switched to manufacture a different product.[8] With single-use technologies, the risk of



A 100-L fermenter located in the fermentation hall at BioVectra's Windsor, Nova Scotia, facility

cross-contamination is significantly reduced. “The regulatory authorities like the technology for that reason, especially on the downstream side,” Dexter says. Regulatory authorities also like the high reproducibility between batches offered by single-use technologies, she adds. “Prepack columns operate pretty consistently from one run to the next.”

The impact of any contamination is also less, Stout says. In the traditional setup, if there is contamination during a chromatography step, multiple batches may have run across it before the issue is detected. With single-use technology, a new column is used for each batch. “With the disposable technology, there’s less risk there because it’s batch by batch,” Stout says.

Single-use technology also allows size matching of the upstream and downstream processes. “Traditionally, the upstream has been either undersized or oversized compared to the downstream. That’s getting better, but with the disposable technology, you have the absolute ability to pair it. You end up getting a very well matched upstream and downstream that processes very well together, and it can process through to drug substance faster,” Stout says.

The disposable tools remain significantly more advanced for mammalian-cell-culture fermentation than for microbial fermentation. End-to-end single-use manufacturing is, however, now possible for pilot-scale microbial fermentations and is planned to be offered at the new BioVectra site.

The bottleneck for the large-scale uptake of entirely single-use microbial fermentation manufacturing processes is upstream: the single-use vessels suitable for microbial fermentations. The largest disposable fermentor is around 1,000 L, whereas 4,000-L bioreactors are used for mammalian cell cultures. “Microbial fermentation is more challenging because of the agitation and oxygen requirements and the heat generated,” Stout says. Microbes grow really fast, generating a lot of heat. As plastic is not a good heat conductor, it’s hard to maintain a sufficiently low temperature inside the reactor, explains Dexter. “That’s why there hasn’t been a scale-up version of it yet,” she says. This isn’t believed to be an insurmountable hurdle. “Some larger disposable microbial fermentation offerings will be in the works in the future beyond the 1,000-L mark,” Stout predicts.

The use of single-use technology is much more established for downstream processing of microbial fermentations. BioVectra intends to offer a hybrid approach, especially for large-scale manufacture, with stainless steel fermentors upstream and single-use technologies downstream. It will also offer clients the option to scale out, not up.[9] Rather than switching to a larger fermentor when demand for a biologic increases, small-volume vessels could be run in parallel. This approach allows for a rapid expansion of capacity.

Again, the scale-out-not-up approach is more established for mammalian cell fermentations, where six-packs of 2,000-L, single-use bioreactors are readily available. “Six 2,000-L single-use bioreactors is equal to a 12,000-L stainless steel reactor with a whole lot less complexity of operation turnover and footprint in

the facility," Dexter says. Scaling out also allows continuity of a manufacturing process from early development through manufacturing, eliminating the need for process optimization for a larger scale.

THE FUTURE

Microbial fermentation is expected to follow mammalian cell fermentation's lead into continuous manufacture. "One of the trends that we're seeing broadly in the industry is the intensification of processes used, [and] one way in which that has become feasible is to think about more continuous operations and production," explains MIT's Love. Each component of biologic manufacture could potentially be engineered to be continuously in operation. Regardless of the industry type, the conversion from batch to continuous processes offers leaner, more cost-effective, and more agile manufacturing.

Two 17,000-L fermenters located in the fermentation hall at BioVectra's Windsor, Nova Scotia, facility

Today, the upstream step of a number of commercial therapeutic biologics is carried out using so-called perfusion mammalian cell culture systems. Here, the cells are retained in the bioreactor and constantly fed. The culture media is then removed continuously from the bioreactor and sent for downstream processing.

"The advancement of technologies in that area has allowed new, smaller facilities for manufacturing [using mammalian cells]. One of the things that we're starting to see now is the emergence of the same concepts applied to microbial fermentation," Love says. BioVectra is taking steps toward this concept and is able to run two fermentors in parallel to create a pseudocontinuous setup. "As you inoculate one fermentor, expand the cells, and transfer into the larger fermentor tank, you can initiate the next ferment for the second tank. You harvest the first, clean, and then prior to harvesting the second, you inoculate and process the first tank on the third batch in a continuous cycle. It's an inoculate-grow-harvest-clean-repeat paradigm to optimize facility utilization, increase productivity, and reduce cost of goods," explains Molly McGlaughlin, director of strategy and business development, biologics, at BioVectra.

Downstream continuous processes have not yet seen large-scale use for biologics, but recent technological advances mean that it is potentially possible and is expected to be seen in use soon.[10] Chromatography is the process for which this technology is most advanced.

Sanofi Genzyme recently demonstrated the feasibility of the ultimate end goal: end-to-end continuous biologic manufacture.[11] Its fully integrated upstream and downstream continuous design can make recombinant monoclonal antibodies from Chinese hamster ovary cells.

In his MIT lab, Love is developing the first end-to-end continuous system based on microbial fermentation. "We've elected to use a yeast organism that has a very clean production profile," he explains. "It's a modular manufacturing system, about the size of a desktop printer, that allows for complete end-to-end production, from inoculation to formulated drug at the end of the process." The production timescale can be as short as three days. "We see future applications for production of small scales of materials for clinical studies or for rare diseases. It could potentially even be used one day in pharmacies or regional manufacturing centers to provide drugs more locally to patients," he says. The project was sponsored by the U.S. Department of Defense, which also foresees the technology being used in the field.

CONCLUSION

Sites for manufacturing biologics from microbes are evolving away from traditional stainless steel equipment toward single-use technologies. This adaptable manufacturing approach can be paired with platform technologies to enable the rapid expansion of capacity, a necessary commodity in today's biologic market. Microbial fermentation methods are expected to follow mammalian cell fermentation toward continuous manufacturing processes. These technological advancements, coupled with the new types of biologics reaching development, mean the outlook for the microbial fermentation of biologics is very bright. "The future for microbial-based products is a very good one," says Dexter.

BIOVECTRA: A REFRESHINGLY ADAPTABLE CDMO

BioVectra is a contract development and manufacturing organization (CDMO) with over 45 years of experience manufacturing intermediates and active pharmaceutical ingredients for global pharmaceutical and biotech companies at its four cGMP facilities. Over the past 15 years, it has had significant successes in the large-scale microbial fermentation of small molecules. More recently, BioVectra has branched into the small-scale microbial fermentation of biologics. "We've been operating at early clinical, Phase I, Phase II projects for biologics," says Scott Doncaster, BioVectra's vice president of manufacturing technologies and engineering.

That biologic capability has now been supersized. The tonnage facility, opening in late 2018, in Windsor, Nova Scotia, houses two 17,000-L stainless steel fermentors. It also contains numerous smaller ferment tanks for higher-titre expression systems and early clinical programs, giving an overall fermentation capacity of 64,000 L.

A platform is planned to be in place by the end of 2019 to rapidly guide biologics from cell-bank development through commercialization of biologics. The hybrid platform incorporates modern cell-line technology and combines stainless steel fermentors with single-use disposable downstream tools. Small-scale single-use fermentors are also an option.

Everything at the site will be contained within modular clean rooms, meaning multiple products will be able to be run concurrently within the facility.

The site will provide a one-stop complete shop for clients looking to outsource the manufacture of enzymes, proteins, peptides, antibody fragments, attenuated virus vaccines, and plasma DNA expressed in microbial systems.

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