

Continuous Bioprocessing and Perfusion: *Wider Adoption Coming as Bioprocessing Matures*

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Introduction

Batch processing has long been the predominant bioprocessing paradigm, both up- and downstream. Bioprocessing fluids are processed incrementally, piped as a bolus or transferred via vessels from one process and piece of equipment to the next. This continues to work well, including a number of technological advances resulting

in improvements that continue to make bioprocessing more efficient. Upstream and overall process yields are essentially doubling about every five years, with this largely driven by improved cell lines, expression systems and genetic engineering, culture media, and equipment. Among the technologies now gaining increasing adoption and market share for biopharmaceutical manufacture is continuous (bio) processing, with perfusion currently the leading technology, in terms of adoption.

The use of incremental, one-step-at-a-time, classic batch processing in biopharmaceutical manufacture is different than most other major products manufacturing and high-tech industries, where processing is generally more continuous. In this context, the move toward more continuous processing in manufacturing is a common characteristic of industries starting to reach maturity. Continuous processing is exemplified by assembly lines, and petroleum refining with processing involving a rather continuous flow of the material being manufactured from one unit operation to the next. Continuous processing generally follows and eventually replaces incremental manufacturing.

Continuous processing generally requires more process knowledge, equipment and technological advances than incremental manufacturing. Successful adoption of continuous processing by any industry requires each of the component processes involved to be more integrated, at least with the next process. Continuous processing requires a sufficient critical mass of technological



competencies and available equipment capable of supporting process integration. For example, implementing continuous bioprocessing, such as upstream perfusion, is not practical if the next and following steps are unable to handle this output.

This article reviews and details some of the key advances and trends in the bioprocessing industry that have

emerged which are creating greater adoption and potential for continuous processing as the industry matures. We evaluate aspects of continuous processing, exemplified by perfusion, and the adoption of these technologies by the biopharmaceutical manufacturing (bioprocessing) industry.

Methods

This analysis includes the review of industry perspectives based on literature, and perspectives from the annual survey of bioprocessing professionals performed by BioPlan Associates, now entering its 11th year.^[1] This survey provides unique perspectives on industry professionals' views. The BioPlan survey involves an extensive online survey and remains the most extensive survey of bioprocessing professionals worldwide. For example, the most recent published 10th/2013 annual survey included responses from nearly 400 individuals, including 238 who work in biopharmaceutical manufacturing, and 158 working for bioprocessing suppliers/vendors, with each group completing customized survey modules.

Findings and Results

Continuous Bioprocessing and Perfusion vs. Batch Processing

Continuous bioprocessing, as it relates to upstream operations, generally refers to perfusion technologies. A generally accepted definition of "continuous" is "the process of running a bioreactor at a fixed volume and

fixed cell concentration for 30–90 days (or longer), with a constant flow of (culture) media through giving a constant harvest volume to be processed.^[2] To separate media and cells, perfusion uses such means as gravity settling, pumping through internal filters, external loop flow-through filters, and centrifugation. Other methods for perfusion involve the host cells, generally selected to be inherently adherent, to microcarrier particles suspended in the cell culture, including magnetic microbeads, with these readily filtered and retained in the bioreactor, and hollow capillary, flat plates, sponge-like matrices, and other fiber- and membrane-based bioreactors, with cells adhering to these substrates during cell culture.

With recombinant proteins on the market since the early 1980s, the biopharmaceutical industry is maturing, with better knowledge and control of component processes, and is incrementally moving toward increased adoption of continuous, now particularly perfusion, vs. batch processing. As discussed below, this generally involves the addition of equipment to conventional bioreactors, so adopting perfusion is not a radical move for the industry.

Fed-batch cell culture has been the dominant bioprocessing method for decades, involving fully loading, running, then emptying a bioreactor. This generally requires cell culture processing within bioreactors for several days to 1–3 weeks. During this time, cells and expressed proteins accumulate at increasing concentrations in the culture medium. This upstream batch bioprocessing is performed such that maximum product yields are obtained, with bioreactor contents vigorously stirred and also often heated, or high metabolic activity requiring cooling. Cell densities up to 100 million/mL can be attained with perfusion, with higher densities correlating with higher product yields. Despite much higher cell concentrations, unlike with batch bioprocessing, there is much less or negligible accumulation of toxic waste products, with proteins on average removed within days of their expression vs. the entire length being retained within fed-batch bioreactors, often for up to 2–3 weeks. With continuous culture (perfusion), lag phases are eliminated because cell culture is always at it or near peak efficiency. Further, the constant flow of fresh media and removal spent media supports operation at higher cell densities.

Between less stress from toxic waste products exposure and less energetic mixing, which contributes to shear that affects cells and proteins, perfusion can generally provide more consistent expressed protein products, generally irrespective of expression systems (*e.g.*, both mammalian and microbial host cells can benefit from perfusion culture). Better expression with perfusion includes higher purity of desired product form, more consistent or desired glycosylation profiles, and less undesired expressed protein

variants—all complications making purification more complex and costly. Perfusion can enable manufacture of less-stable product, due to shorter bioreactor residence time. In fact, for some products, processes, and host cells, perfusion may be the only option, with conventional fed-batch bioprocessing simply not providing sufficiently high quality product or requiring excessively large bioreactors and other equipment.

Commercial manufacture generally involves using bioreactors over 1,000–2,000 L, and this requires large-scale industrial operations and equipment. Obtaining the same amount of product more continuously involves smaller bioreactors and other operations—mixing, heating, cooling, bulk transfers, *etc.*—at smaller scale. And if problems do occur with perfusion, only that part, the media and product removed during the process run when problems were encountered, need be discarded. In contrast, with fed-batch bioprocessing, the entire batch/lot must be discarded.

Continuous bioprocessing at the industrial scale has long been a goal for bioprocessing.^[5-7 3-5] Perfusion, the current leading continuous bioprocessing technology (in terms of adoption) was unattainable in the 1980s. Expression yields were too low, culture media too primitive, and the technologies and equipment simply could not be integrated as needed. In the 1990s, industry largely moved away from implementing perfusion as new expression systems, culture media and supplements, and other advances contributed to dramatic progress with batch processing, including yields doubling about every five years. Fed-batch bioprocessing remained familiar and efficient, while perfusion had problems including complexity and higher failure rates. A major exception was widespread adoption in the 1980s of cell-adhering hollow fiber-based bioreactors for manufacture of monoclonal antibodies, with these used with classic hybridomas, not conventional recombinant (*e.g.*, genetically engineered CHO cell-based) monoclonal antibody manufacture, which displaced this in the 1990s. Now, many are predicting that “perfusion will make a comeback as the manufacturing method of choice”^[6 4] or otherwise see significant advances and adoption in coming years.^[3,4,7 3-5]

Adoption is driven by economics. Continuous bioprocessing manufacture is generally cheaper, with reduced costs of goods and capital investments (*e.g.*, smaller-scale facilities). Associated with this, it is generally simpler, requires less infrastructure, utilities, staff, uses smaller and less expensive equipment, also it is milder (less energetic), controllable, and steady, compared to batch mode-based manufacturing. Bioreactor harvest is withdrawn continuously, allowing purification to be done more continuously and/or repetitively at smaller scales. Handling all the process material at once, batch processing requires larger bioreactors and other equipment that cost more, take up

more space, are more labor intensive, and require a more robust industrial infrastructure. Plus, conventional batch processing is by nature, intermittent, sporadic, and uneven, which contributes to inefficiencies and complicates planning. Continuous bioprocessing allows more predictable, steady manufacture of the same (or even more) amount of product in bioreactors and other equipment at smaller scales with associated costs-savings and benefits.

Advancing Technology Trends for Continuous Bioprocessing

Single-Use Bioreactors: Significant advances have taken place in bioreactors, especially in single-use/disposable bioreactors and other equipment, and they now dominate small- and mid-scale bioprocessing. The markets for single-use bioprocessing systems will increase significantly in the next five years.^[8] This growth is driven by products being developed using single-use systems receiving approvals and graduating to commercial manufacture. Only relatively recently have cost-effective perfusion systems become available in single-use format. This includes perfusion technology adoption such as the alternating tangential flow-based ATF System™ from Refine Technology, involving a pump and filter to retain cells within bioreactors with continuous outflow of culture media.^[2]

High Yield Cell Lines: Ever-improving high-yield cell lines, expression systems, and optimized culture media are providing more platforms for development and adoption of continuous bioprocessing, exemplified by upstream perfusion.^[3-7]

Other Technologies: Continuous upstream bioprocessing, particularly perfusion, is currently experiencing increasing adoption. Products manufactured using perfusion bioreactors include Kogenate® (factor VIII) from Bayer; ReoPro® (anti-platelet mAb) and Remicade® (tumor necrosis factor mAb)

from Janssen/J&J; Campath® (CD52 mAb) from Genzyme Sanofi; and Xyntha® (modified factor VIII) from Pfizer.^[8]

Other perfusion options are seeing slower adoption: centrifugation, conical or inclined cell settlers, spin filters, use of cell microcarriers to retain or filter and return cells to the bioreactor, and binding of cells to fiber- or membrane-based bioreactors. The adoption of continuous downstream purification is proving more difficult, and is lagging behind. Continuous chromatography methods, such as simulated moving bed (SMB) and periodic counter-current chromatography, are generally not yet ready yet for widespread adoption.

Cost and Yield Estimates

Using Refine Technology's ATF System to estimate costs, at the 500 kg/year level, annual bioprocessing expenses with single-use equipment are projected at \$33.1 million for perfusion and \$106.7 million for fed-batch. Comparing stainless steel-based costs, we find \$44.1 million for perfusion and \$103.9 million for fed-batch manufacture.^[5] Assuming that cost savings such as these are broadly applicable, perfusion technologies will probably increase in implementation. As with most biomanufacturing, these will typically be for new bioprocesses, as retrofitting existing GMP processes tends to be substantially more difficult.

Generally, perfusion provides more product than fed-batch manufacture over the period of time of perfusion operations, even when using smaller bioreactors. For example, CMC Biologics, a contract manufacturing organization (CMO) now offers both perfusion and fed-batch processing, and has reported studies comparing the two methods, with perfusion favored in many aspects.^[4] One product evaluation gave a fed batch culture yield of ~55 mg/L/day after 12 days, while perfusion, over the same period, provided ~425 mg/L/day, providing an order of magnitude more product (Figure 1). Other companies have

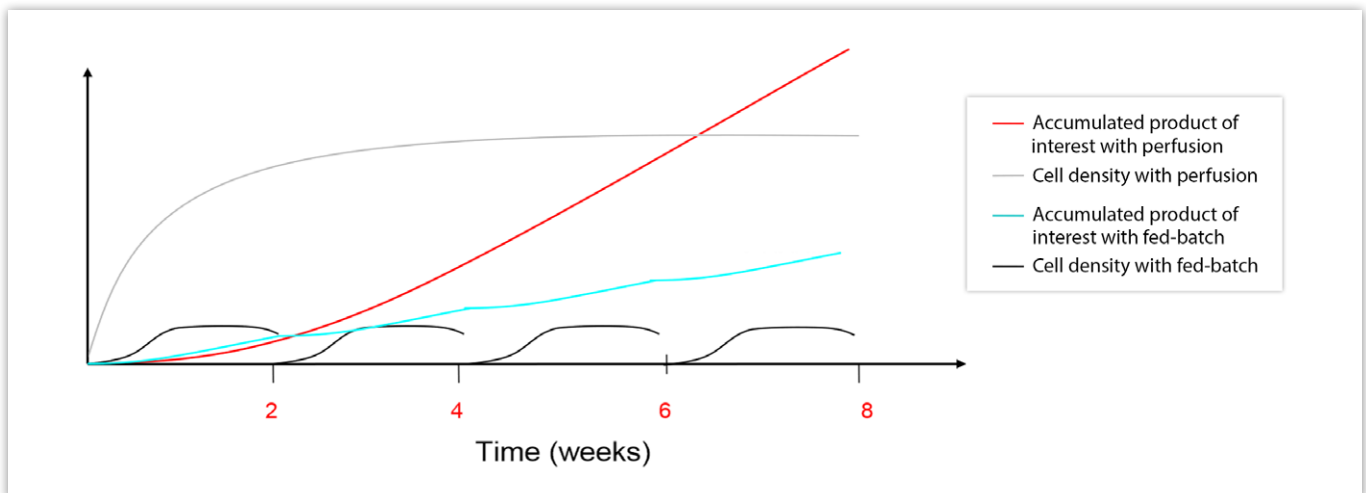


FIGURE 1. Perfusion vs. fed-batch product expression.^[6] (Used with permission from Carstens *et al.*)

reported benefits from perfusion. For example, Bayer AG reported “our specialty is reliable, high-throughput continuous perfusion fermentation. Our in-house-developed inclined plate settlers allow us daily harvesting of 200–500 L from one single 100 L fermenter. This yields in multiple kilogram quantities of monoclonal antibodies from one unit (bioreactor).”^[8]

Depending on the methods and equipment used, perfusion can involve much less or more culture media than comparable fed-batch production. But in general, longer, slower perfusion, at higher cell concentrations, over the same extended period beats batch manufacture.

With batch processing, product-related quality problems tend to be more common, because the product remains in the bioreactor much longer, exposed to stressful conditions (e.g., mixing and heating) and by-products (e.g., lactic acid and ammonia) that can be toxic to cells as levels increase. With perfusion, these contaminants are continually removed and replaced. At lower scales, perfusion allows the use of lower-yield cell lines, reducing cell line, bioprocess, and culture media optimization, providing speed and costs savings. But by far, the benefits with perfusion accrue fastest at commercial manufacturing scales. This is because commercial-scale processing generally runs over many years, unlike clinical-scale production, which is sporadically performed.

Problems with Continuous Bioprocessing Slowing Adoption

Wider and more rapid industry adoption of continuous processing, exemplified by upstream perfusion, has, until recently, been restricted by a lack of suitable technologies and equipment. But as technologies advance, some of the factors holding back wider adoption include misperceptions and lack of knowledge within the industry, as has been reported in this journal.^[5] It simply takes time for most changes in bioprocessing to occur, with this highly-regulated industry being rather slow to adopt new technologies, and rightfully concerned over truly novel approaches that may raise safety or other concerns with industry regulators. Another aspect holding back perfusion is bioprocessing modeling, because most modeling software is batch-based, and not for continuous processing where there is limited experience data.^[10] This source also notes that perfusion is more complex to model, which also provides

greater opportunity to optimize production.

The Annual Survey of bioprocessing professionals by BioPlan Associates has documented the industry’s concern over process complexity. In our 2103 10th annual industry study, we evaluated perfusion issues.^[1] From this survey (as shown in Figure 2), a number of problems were consistently associated with perfusion vs. fed-batch, despite equipment manufacturers and users reporting these

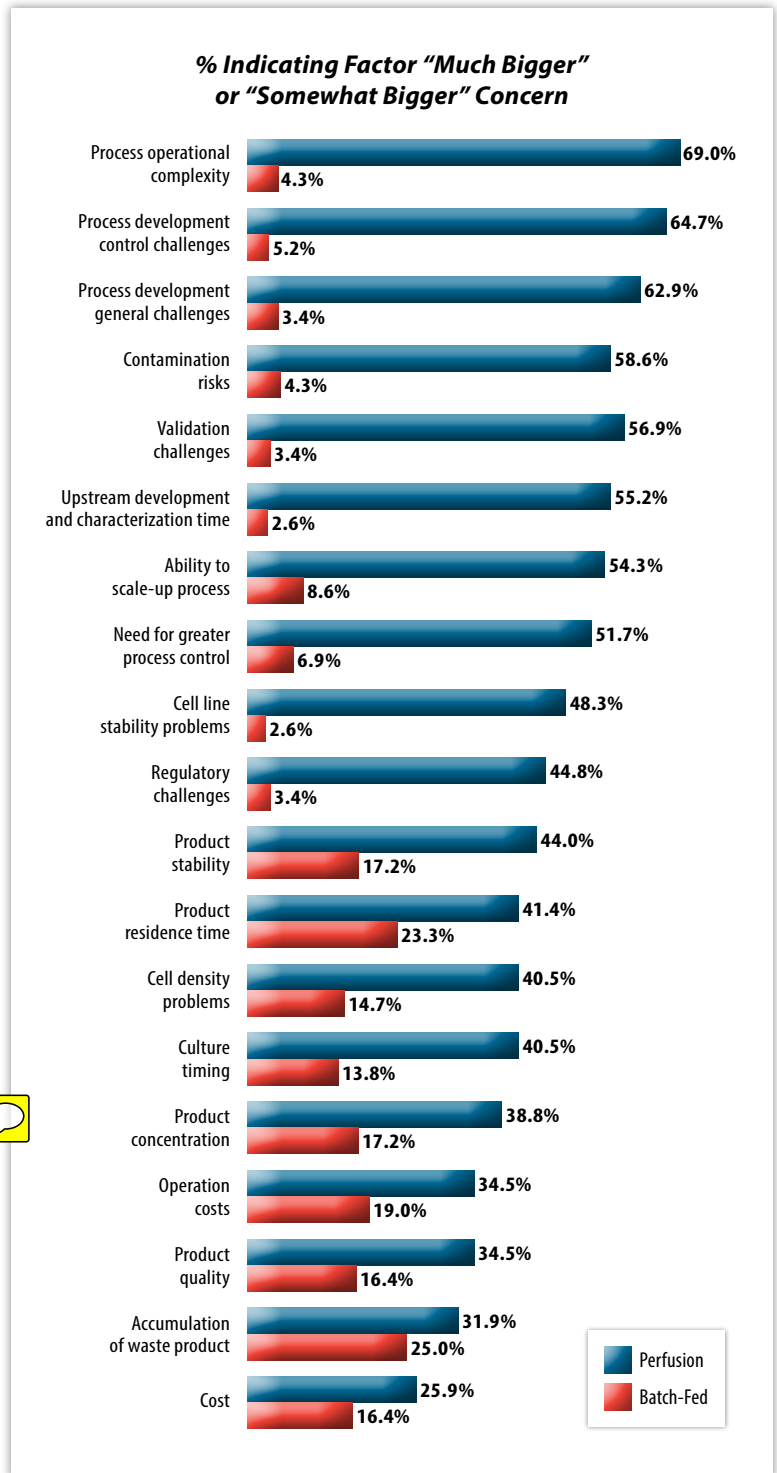


FIGURE 2. Perfusion operations issues: perfusion vs. batch-fed processes.^[1]

problems having been resolved. Given a list of 19 problems encountered in bioprocessing, respondents consistently rated all as significantly more serious with perfusion than with batch processing. For example, “process operational complexity,” “process development control technologies,” and “process development general challenges” were each cited a problems by over 60% of respondents compared to 3–5% for batch processes. These percentages of respondents reporting problems with perfusion vs. fed-batch bioprocessing were higher in 2013 than in the last survey in 2010 which asked this same question (*i.e.*, perception of problems with perfusion). Problems have actually increased in recent years, according to our most recent survey.

But perfusion is now generally less complex, with many systems relatively simple “bolt-on” units, less prone to contamination, and more readily scalable than fed-batch methods. Fears that regulators will perceive problems have proven less relevant, including difficulties in defining lots/batches and doing QA/QC with continuously manufactured products, with FDA now encouraging continuous processing. Perfusion equipment manufacturers, obviously, seek to make it easy to adapt their equipment to existing bioreactors and those being planned. Most conventional bioreactors, designed for batch operations, can have an alternating tangential flow system, specialized filters, centrifugation, or other perfusion equipment readily incorporated in up-front design. And many, or most, existing fixed stainless steel bioreactors can be adapted to perfusion operations.

The Future for Perfusion and Continuous Bioprocessing

Advances in bioprocessing knowledge, equipment, monitoring technologies, automation, cell retention systems, and single-use technologies have all made the capability of running large-scale (≥ 1000 L) perfusion less risky. Yet, there continues to be a knowledge gap as most in the industry continue to perceive perfusion with creating additional complexity. This is changing as evidence of performance increases. A new facility can run several 500 L bioreactors continuously (at 2–3 g/L/day) and link to a small, continuous downstream train, producing ton quantities of a desired antibody per year.^[† 22]

Although quantitative market data are not available, companies offering perfusion technologies are reported to be seeing increasing sales, including scales of perfusion operations increasing as products progress in the development pipeline. Adoption of perfusion and upcoming downstream options for more continuous purification will see steady incremental adoption in coming years. New technologies are in development, such as involving centrifugation and tangential filtration, which will also see

increasing acceptance.

New perfusion technologies could even revolutionize certain types of bioprocessing. Take, for example, a 50 L desk-sized permeable hollow capillary fiber-based bioreactor, with cells bound to the fibers, in development by FiberCell Systems. It is projected to manufacture the same (or more) quantity of product, over much the same time as conventional multi-batch manufacturing campaigns, at better quality, comparable to a 1,000–5,000 L fed-batch bioreactor using the same amount of culture media, same host cells, *etc.*^[4] Similar fiber cell-binding bioreactors were in common use for now-classic (legacy) fused-cell hybridoma-based monoclonal antibody (non-recombinant) manufacture back in the 1980s. So in this context, current increased adoption of perfusion could be viewed as a comeback, rather than being fully novel or revolutionary. Other current manufacturers of fiber- and membrane-based perfusion bioreactors include ZellWerk, represented by Glen Mills in the U.S., Biovest, and ATMI. Other offering perfusion-oriented bioreactors include PBS (Refine Technologies), Xcellerex and Wave Biotech (GE Life Sciences), and <<Is it BioSep?>> (Applikon).

Perfusion is increasingly being accepted by regulatory authorities, assuaging many concerns that regulators will react negatively to this new technology. This has even included Dr. Janet Woodcock, Director, FDA/CDER, predicting the obsolescence of batch processing in favor of continuous processing approaches to biopharmaceutical manufacturing.^[10]

However, perfusion and continuous bioprocessing, in the long term, will likely not become the predominant bioprocessing paradigm just yet. Batch processing is very well-understood, and well-accepted, and most equipment still oriented to this. Most bioprocessing professionals are trained and experienced with batch processing but still lack hands-on experience even with increasingly common perfusion. Batch processing remaining simpler to design and implement, and regulators in many countries other than major biopharmaceutical markets (*e.g.*, U.S. and EU) are still unfamiliar with perfusion. In general, the industry is hesitant and slow to adapt novel biopharmaceutical product manufacturing technologies. Advances in expression systems, genetic engineering, cell lines, bioprocess design and modeling, culture media optimization, metabolic engineering, *etc.* will continue to boost cell-specific productivities. Simple batch processing will continue to see incremental increases in volumetric titers and yields, becoming more efficient. Thus, perfusion and continuous bioprocessing will have serious competition for many users. Perfusion tends to be adopted where capital and facility investment expenses and a lean operation at smaller scales are primary decision-driving factors.

Conclusion

Much as with single-use systems over the past decade, continuous bioprocessing and, in the near term, upstream perfusion, will see increasing adoption. This includes large-scale and commercial biomanufacturing. Much of this will be with single-use perfusion equipment, particularly, as current clinical-scale biologics being developed by single-use manufacture graduate to commercial scale. But with the operational efficiencies offered by perfusion, with many currently available technologies being bolt-on or interfaced with current stainless steel bioreactors, an increasing number of established fixed stainless steel-based facilities will retrofit for perfusion operations. Further, many current and future hybrid facilities (with a mix of single-use and stainless steel equipment) can be expected to adopt perfusion.

As new downstream, continuous processing technologies start to become available, it will ultimately become feasible to perform full end-to-end bioprocessing, both up and downstream. Once this happens, the economics of continuous bioprocessing can be expected to be further favored when compared with batch processing. We will likely also see more rapid adoption as the industry (and leaders such as Genzyme/Sanofi, Amgen, and GlaxoSmithKline) continue to adopt perfusion and report success in improved efficiency, lower costs, and better quality products.

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