Commentary

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Single-use technology supporting the comeback of continuous bioprocessing

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As advanced single-use biomanufacturing solutions are contiguously incorporated with single-use perfusion mode-capable reactors, the design of vertically integrated, closed, disposable and continuous upstream bioproduction systems is finally being realized.

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By many measures biotechnology recently celebrated its 30th anniversary. The current dominance of the fed-batch culture of suspension cells in manufacturing of biopharmaceuticals often obscures its robust history of process development. One interesting theme, for protein biologicals especially, is the waxing and waning of a variety of approaches to continuous processing. In fact, a number of implementations of this mode of production have long been in use by such well-known pharmaceutical manufacturers as Genzyme (Sanofi) and Centocor (Johnson & Johnson/Janssen) to make highly successful products, such as Cerezyme® and Remicade®. Interest in continuous bioprocessing (CB) is growing of late due to a number of economic, technological and regulatory developments. Features provided by single-use bioproduction systems complement those provided by CB methods, and the number of operations supported by them is large and growing.

Continuous process manufacturing

Continuous processing (CP) is a mode of manufacturing in which raw materials continually flow in and out of equipment as they are processed into an intermediate or final product. In pharmaceutical manufacturing, this process is generally accomplished in a chemical- or bio-reactor. The CP method is in contrast to discontinuous 'batch' production in which a specific quantity of product is produced in a single, discrete volume during the same cycle of manufacture. Batch production is frequently segmented into many individual steps that are often performed at separate facilities (e.g., suites, buildings or cities). In CP, production occurs at a single location and without interruption. It proceeds in that way for variable lengths of time – from days, to weeks, to months – and is only interrupted for reasons such as cleaning of equipment or the incremental deterioration of such constants as catalysts or cultures.

Demonstrable improvement of manufacturing processes through conversion from batch to CP methods began in the late 19th century and has continued unabated in a diverse range of industries, including the production of steel, petrochemicals and foods. The advantages CP methods generally provide, even to such dissimilar products, include: steady-state operation, reduced equipment size, streamlined process flow and reduced capital costs [1]. The food industry, for example, proudly promotes its linear factories in which materials are continually trucked in at one end as product comes out the other.

So, if so much is to be gained, and since it has been considered for so long, why has

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its acceptance by biopharmaceutical companies been so delayed? Reasons for the relatively slow penetration include a previous lack of understanding of critical process parameters in bioproduction; a lack of, difficult and slow process monitoring techniques; a general low tolerance to risk; management reluctance to new technologies; over-investment in facilities designed for batch; the immediate economy of fully depreciated plants; and a perception of regulatory constraints [2].

While this reluctance to change has been, until recently, particularly strong in the field of biopharmaceuticals – both an evolving business environment as well as new regulatory imperatives are changing this. The biotech industry is now seriously pursuing CP methods. Examples of the current interest in the (bio) pharmaceutical industry include: the Novartis–MIT Center for Continuous Manufacturing, which is addressing a radical redesign of historical manufacturing processes to achieve fully vertically integrated continuous flow [3]; and a number of international conferences either including, or even solely dedicated to, the topic of CB.

Continuous processes in upstream bioproduction

The advantages most often touted for continuous production in bioprocessing include lower initial investment costs, lower facility and operating cost, reduced operator requirement, as well as both design and operational speed, efficiency and flexibility.

By far the most common approach to continuous processing in upstream animal cell-based bioproduction is through perfusion culture [3,4]. In perfusion culture, medium is added at rates exceeding the cell mass expansion rate and the excess medium is removed using some device to retain cells in the bioreactor [5]. A number of such research- and production-scale perfusion bioreactor systems have been devised [6]. Although many perfusion processes for either suspended or adherent animal cells are known to be used in manufacturing-scale biopharmaceutical production, details on their design and operation are not always publically available. Terminology in this dynamic field can get fuzzy – for example, CP is also referred to as continuous production, continuous flow processing or continuous manufacturing, with minor distinctions sometimes made between them. Depending on the periodicity of either entire production episodes, or of more discrete individual component operations, some even apply such terms as semi-continuous or pseudo-continuous operation [7]. Nevertheless, interest in CB is growing [8–10], significant stakeholder investment is occurring [11] and commercialized instrumentation to support its incorporation in single-use (SU) or hybrid applications is now appearing.

CB concerns & inhibitions

Justified or not, specific concerns have been expressed regarding the implementation of CP in bioprocessing. These concerns include performance reliability (incidence of failure), validation complexity, process control and economic justification [12–14]. But for many processes, such previous limitations – or their perception – are being alleviated by specific advances in CB processing technology or by OpEx-driven advances in bioprocess understanding, reactor monitoring and feedback control. Concern for limited bottom-line financial savings in CB has been countered by this possibly being a small-molecule issue only, due to the limited contribution of active pharmaceutical ingredient manufacturing costs to total small-molecule pharmaceutical costs. Furthermore, it is argued that biopharmaceuticals is a different animal in general, and as trends such as globalization and biosimilars alter the picture even further, the financial benefits of CB will become even stronger [15].

Nevertheless, while some CB attributes inherently provide immediate advantages (such as reduced reactor residency time) others do present challenges (such as cell-line stability concerns). The latter issue is that no matter how a perfusion reactor is maintained, if cells are dividing, the number of generations from original cloning or validation is increasing. This introduces at least two distinct issues: first, many cell lines deteriorate in some way(s) after some tens of generations from production, and second, even if productivity or product quality is unaffected by generation number, this fact would have to be validated for the duration term of continuous production.

» SU in bioprocessing

SU in bioprocessing refers to materials or equipment intended to be used in a single processing batch or campaign, usually having a product contact surface element that is disposable. Such equipment ranges from single material, very simple stand-alone items such as a tubing, to complex and controlled systems of many components and materials, such as a bioreactor [16]. Relatedly, the application of such equipment ranges from an instrument with a single, simple function, to applications housing entire – or even combined unit – operations. Most of the more complicated SU systems contain reusable non-product-contact elements, for such purposes as support. SU systems have been taken up in the biopharmaceutical industry in general because of benefits such as reduced contamination risks; reduced time to market; heightened ease of use; lower initial investment and facility and operating costs; reduced operator requirement; and a general processing speed, efficiency and flexibility.

Over the past 10 years, the number of individual SU process activities, as well as entire systems, available has grown substantially and now include cell culture for seed expansion and production; media and buffer preparation, transport, storage; liquid pumping, filtration, collection, shipping; on-line and in-line contents sensing, sampling and analysis; and the cryopreservation of culture seed and product intermediates. Some of the newer items available for upstream applications include disposable pumps, SU flowpath autosampling and microcarrier separators [17].

» SU in CB

SU technologies offer a number of values to any mode of bioprocessing, but can provide some specific and enabling features in CB implementations [18,19]. Almost every operation in a CB process train is now supported by a commercially available SU, or at least hybrid, solution. First of all, many of the SU equipment and solutions being developed for batch bioproduction have the same or related application in CB systems. Examples here include simple equipment such as tubings and connectors, to more complex applications such as the cryopreservation of large working stock aliquots in flexible bioprocess containers (BPCs). The list of CB-supporting SU technologies being developed is large and growing. SU supports early upstream activities such as the preparation and storage of media or buffers in SU mixers, and their storage for CB feeding in SU BPCs. Around the CB reactor itself there are SU liquid and gas filtration materials of many types. There are SU products supporting the metered distribution of liquids through SU manifolds as well as the storage and distribution of dry powders. SU or hybrid bioreactors support cell culture in seed generation and for actual production in a variety of perfusion capable bioreactors. There has been contined appearance of new SU monitoring probes and sensors of various styles and analyte targets. There are vendors of commercially available SU apparatus for SU sampling in real-time for automated, online, multi-analyte monitoring. Online, real-time controlled sterile reactor feed porting provides SU input of any required solutions during production. SU flow-path bulk harvesting can be accomplished by SU centrifugation or filtration into SU BPCs of a variety of styles. Downstream, purification can be accomplished in SU traditional, hollow fiber, membrane or PCC (simulated moving bed) chromatography. Final fill is now supported by a number of SU flowpath and automated and/or closed apparatus.

A SU advantage in CB process development is its support of an open architecture approach and a number of hybrid designs. Such designs include combining reusable and SU systems, or between divergent suppliers of particular equipment. Especially in bioproduction, the many flexibilities of SU support a manufacturing platform of exceptional efficiency, adaptability and operational ease. Advances in designs of SU transfer tubing, distribution manifolds and container porting also supports creativity in process design. This is of particular value in designing a process with such demands as an entirely new flow path, monitoring or lot designations – such as for CB.

SU systems upstream provide a reduced footprint and eliminate the need for cleaning and sterilization service. This complements perfusion culture's inherently smaller size and independence from cleaning for extended periods of time. Several newer approaches to formulating process fluids support the concept of CB. SU mixing systems are typically constructed of a rigid containment system with a motor and controls driving radiation-sterilized SU bags equipped with disposable impeller assemblies. From a variety of manufacturers there are a number of distinct approaches to motor/disposable impeller assembly linkages, tubing lines and connections. Also appearing are a number of exciting SU sampling, sensing, and monitoring solutions. SU powder containers permit seamless transfer between powder and liquid formulation steps, and the mixing container supports are available in jacketed stainless steel for heating and cooling requirements. Surprisingly, the 'topping-up' of large-scale SU fluid containers with newly prepared buffer to provide a virtually unlimited and constant supply of each buffer/media type can be validated for GMP procedures.

Continuous, automated in-line culture media and buffer dilution and conditioning have been attempted for decades, and interest in them remains high. Advancements in the mass flow technology, monitoring and feedback control required to establish and maintain process fluid specifications are now allowing such approaches to become a reality. The compact size and portability of the equipment involved allows it to produce fluids at the 'point of use' and is supported by the incorporation of SU. Thus, in-line fluid preparation and conditioning provides benefits to bioprocessing in general, supports CB in particular and provides specific features supporting SU technology application in CB. For example, its significantly reduced buffer prep tank size requirement supports application of SU BPCs and manifolds.

Process flexibility is a key feature in both SU and CB. CB contributes to overall process flexibility in that equipment tends to be easy to clean, inspect and maintain – and generally promotes simple and rapid product changeover. SU systems can provide similar flexibility and ease product changeover because they tend to be more modular and transportable than much

of the older batch equipment. In fact, the size, configuration and reduced service requirements of SU systems actually encourage diversity of physical location within a suite or plant, as well as relocation to other manufacturing sites.

Due to its inherent demand for immediate process data and control capabilities, CB supports initiatives in continuous quality verification, continuous process verification and real-time release. Although CB will not be feasible for all products and processes, many implementations well-support a 'platform' approach in which a single process supports more than one product. CB nearly always shortens the process stream, reduces downtime and greatly reduces handling of intermediates. These features complement the operational efficiencies of SU systems, contributing to a greatly reduced cumulative processing time for the active pharmaceutical ingredient. Furthermore, they greatly simplify production trains and inherently facilitate application of closed processing approaches to individual operations and even processes. Especially in bioproduction, the modularity and integral gamma irradiation sterility of SU combined with the sustained operation of CB promise the appearance of platforms of unparalleled operational simplicity and convenience. Advantages here go beyond convenience and speed in production of one product, and open up the possibility of manufacturing of divergent product types in suits of reduced environmental classification.

The heart of a CB approach is the bioreactor. Perfusion bioreactors have been successfully employed in bioproduction, even biopharmaceutical production, for decades. At the research scale there have even been SU hollow fiber perfusion bioreactors available from a variety of vendors for over 40 years. However, only recently have commercially available SU and hybrid production-scale perfusion-capable equipment appeared [16,20]. Such systems are composed of SU and hybrid perfusion-capable reactors; a growing variety of SU and hybrid monitoring probes and sensors; SU pumps and fluid delivery automation of various design; and automated SU online sampling, interface, valving and feeding technologies. Their coordinated implementation in actual production settings with appropriate control is now beginning.

The fact that many SU systems are constructed from materials that are compliant with pharmaceutical standards and are free from animal products, supports CB applications in a wide variety of product types and classification. In fact, SU systems are available to most any process format (e.g., microcarriers and suspension), platform (e.g., cell line, vectors, culture media), mode (e.g., dialysis or intensified perfusion) or scale (e.g., through rapid, inexpensive horizontal scale-out). 'Future proofing', or supporting the sustainability of a new CB process in the face of product lifecycle or emerging technology imperatives, is supported by many SU features. Examples here include low initial facility, service and equipment costs – and especially, the undedicated manufacturing suits and ease of process train reconfiguration.

CB has, however, introduced an interesting twist on the standard paradigm of the concept of iterations of equipment usage. There has always been a bit of wiggle room in the distinction between the concept of 'single-use' and such terms as 'disposable' or 'limited-use', and it is important to consider how CB has determined a re-examination of a few related concepts in this regard. For example, in CB one may employ a piece of equipment or material 'once' for many weeks or months, which had been originally designed to be used 'once' for a matter of hours or days.

» Regulatory commitment

It has been 10 years since the US FDA articulated, in its process analytical technology guidance, the goal of "*facilitating continuous processing to improve efficiency and manage variability*" in pharmaceutical manufacturing, and CP implicitly supports many quality-by-design goals. Janet Woodcock (Director of the FDA Center for Drug Evaluation and Research) once commented that "*continuous manufacturing is going to become a reality, not just as a high-tech way of doing things, but as a successful, competing, disruptive technology that will move into the industry and really change the way things are done.*" She said this is because it will be "*adaptable, flexible, hugely more efficient, less polluting and easily replicated.*"

Regarding the mode of manufacturing to be used, most relevant regulations and guidance are either silent, imply flexibility or specifically allow the CP mode. Drug product produced by a continuous process is specifically defined in 21 CFR 210.3(b). The FDA has recently specifically addressed the need to "*investigate the effects of continuous manufacturing (manufacturing using a continuous process, rather than a batch approach) as a means of improving product quality.*" The International Conference on Harmonization notes in its Q7 guidance, *"In the case of continuous production, a batch may correspond to a defined fraction of the production"*, and the International Pharmaceutical Excipients Council states "*For continuous processes the batch and its records should be defined*". The European Medicines Agency referred to CP in its draft Guideline on Process Validation, and the FDA released a strategic plan, Advancing Regulatory Science, which includes an advocacy of CP. CB is also supported by the European Medicines Agency's recently released guideline on real

time release testing and the European Commission's 'Factories of the Future'.

Conclusion

As advanced SU biomanufacturing solutions are contiguously incorporated with SU perfusion mode-capable reactors, the design of vertically integrated, closed, disposable and continuous upstream bioproduction systems is finally being realized.

Financial & competing interests disclosure

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