Single-use perfusion bioreactors support continuous biomanufacturing

The field of biomanufacturing employs many single-use (SU) systems for features such as lower initial investment and reduced time-to-market. Continuous manufacturing methods can provide increased product quality and process control, and reduced operating costs. Continuous biomanufacturing (CB) additionally promises reduced classified area extent, personnel requirements and operating steps. The benefits of SU CB include heightened processing parameter consistency and increased process flexibility. Perfusion culture (PC) is supported by many specialized SU and hybrid bioreactors. These and many accessory perfusion-enabling technologies are the main way of implementing upstream CB. Valuable features provided by PC include reduced product reactor-residency and increased reactor-volumetric productivity. SU PC supports the dream of enterprise managed, modular, end-to-end integrated and closed CB providing higher environmental sustainability.

Single-use systems

The biopharmaceutical industry now incorporates significant levels of single-use (SU) technology and systems in the majority of animal cell culture-based production processes [1,2]. Implementation of these technologies has resulted in prepackaged and sterilized systems, complete and ready for use with preinstalled impellers and monitoring probes (Box 1) [3]. SU bioreactors (SUBs) are now available from multiple suppliers, with several offerings of up to a 2000 l working volume [4–6]. A variety of sparging/mass-transfer and cell suspension/segregation designs are available – from innovative packed bed to rocker-style, top or bottom-mounted mixing devices, orbitally shaken or even ‘air-wheel’ impelled reactors [7].

From upstream process material preparation through final product formulation, biopharm sponsors are increasingly presented with numerous SU solutions supporting all major production platforms [8–10]. SU flow-path centrifuges, depth and diafiltration (including tangential flow filtration [TFF]) and heat exchangers allow disposable down-stream processing to begin directly from the bioreactor harvest [11]. While existing systems for disposable processing have been accepted in bioproduction for many operations, new developments in the technology and scale of application continue to be presented. This includes SU applications in vaccine manufacturing [12,13] and cell-based therapies [14–16]. Exciting advancements continue in the areas of fluid connectivity and reactor component sampling and monitoring [17]. While SU-based bioproduction has traditionally been applied to animal cell-based production, an increasing number of system suppliers are supporting manufacturing-scale microbial fermentation. While such applications are just beginning, it is anticipated that in the near future, SU fermentation of yeast, bacteria and fungi will become commonplace.

Off the shelf, SU systems are now in regular use to some extent in nearly every segment of the production train by contract manufacturing organizations (CMOs) and sponsors in regulated production applications [18–20]. The field has progressed to the point that there now exist pre-engineered, modular, modular microenvironment and turn-key facilities [21]. Such futuristic facilities can...
employ areas of discrete and limited classified space (controlled environmental modules, modular microenvironments, isolation chambers or dedicated isolator cabinets) or even within prefabricated trailers or pods [22, 23]. As this trend toward flexible bioprocessing modules and plug-and-play factories continues, we anticipate such facilities to become available with higher-level integrated system management [24, 25]. For a number of reasons, including component availability, process unit operations or even individual instrumentation composed of both single-use and reusable product contact components (hybrid systems) remain quite common.

### Continuous processing

Continuous manufacturing processes proceed for variable lengths of time – from days to months to years – and are only interrupted for such reasons as cleaning of equipment or the incremental deterioration of enzymes, catalysts or cultures. This is in contrast to the discontinuous ‘batch’ production, where a specific quantity of product is manufactured in a single, discrete volume during the same cycle of manufacture. Another distinction is that batch production is frequently segmented into many individual steps, often performed at separate facilities (suitees, buildings or cities) with significant effort involved in intermediate hold and transport, whereas continuous processing (CP) occurs uninterrupted at a single location.

It should be considered that terminology in this dynamic field can sometimes get fuzzy – for example, continuous processing is also referred to as continuous production, continuous flow processing or continuous manufacturing. Furthermore, minor (to nonspecialists) distinctions are often made between different implementations and styles. In fact, few processes are absolutely and exquisitely ‘continuous’ in a strict sense, and contemporary definitions often allow for some degree of continual, multiple discretation of the process or output. Depending on the periodicity and duration of entire production episodes, or the specific nature of more discrete individual component operations, some apply such terms as semi-, pseudo- and quasicontinuous, hybrid or even microbatch based processes. Nevertheless, the characteristics and values described here do apply to many such incompletely continuous processes.

### Continuous biomanufacturing

Interest in the use of CP techniques in large-scale biomanufacturing has increased dramatically in the past few years, to the extent that many are now predicting its eventual dominance in the industry [26, 27]. This is due to the many benefits either CP itself, or the process intensification operations contributing to it (see Table 1) [28]. Continuous biomanufacturing (CB) is encouraged by pharmaceutical regulatory agencies and provides many specific benefits in bioprocessing [29]. A growing number of biopharmaceutical manufacturers currently employ continuous processes in unit operations, and recent developments promise to stimulate even more interest in them [30].
Quite a number of culture mode and process options that can accommodate a CP approach have existed for decades. Recently, a number of manufacturing-scale perfusion or perfusion-capable bioreactors have been launched (Table 2), and this includes the currently most popular stirred tank single-use bioreactors (SUBs). Successful CB implementations have now been achieved in a number of good manufacturing practice (GMP) installations of premier biopharmaceutical sponsors, including for approved products. For example, Genzyme’s continued commitment to perfusion-based production is demonstrated by an expansion of such perfusion cell culture capacity at their Geel, Belgium plant involving 4000-l perfusion bioreactors, with dedicated seed and purification trains.

CB is further enabled by ongoing technological developments in mixing and mass-flow systems and (even more importantly) by the many recent advances in process monitoring and feedback/feed forward manufacturing process control [33]. It should be noted that CB efficiently promotes both the tools and goals of operational excellence, thereby enabling such initiatives as PAT and QbD [32,33]. It readily accepts many new in/on-line monitoring approaches, real-time quality assurance as well as developments and advances in process automation. Combined with the industry’s growing process understanding, CB extends such capabilities to advancing continuous quality verification (CQV), continuous process verification (CPV), and real-time release (RTR) enabling initiatives. Although CB will

<table>
<thead>
<tr>
<th>Feature or attribute</th>
<th>Example or benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endorsed by EMA guidelines and US FDA PAT guidance</td>
<td>Provides guidance in process design and ease of filing</td>
</tr>
<tr>
<td>Supports advanced automation; on/inline monitoring</td>
<td>Reduces operator costs and manual control errors</td>
</tr>
<tr>
<td>An old, well-established technology and engineering</td>
<td>From cracking oil to cooking biscuits to distilling fluids</td>
</tr>
<tr>
<td>Accepts materials/chemistries unavailable in batch</td>
<td>For example, residence time-sensitive reactions now considered</td>
</tr>
<tr>
<td>Promotes QbD tools; heightened process understanding</td>
<td>Provides robust operation with higher product quality</td>
</tr>
<tr>
<td>Lot determined by run time and not containment size</td>
<td>Greatly reduces process and reaction mass and volume</td>
</tr>
<tr>
<td>Accommodates remarkable process intensification tools</td>
<td>Further increases reactor-volumetric productivity</td>
</tr>
<tr>
<td>Reduces reactor size and classified area footprint</td>
<td>Supports single-use and scale-based numbering-up</td>
</tr>
<tr>
<td>Provides modular, mobile equipment and processes</td>
<td>Increases suite flexibility/ eases product changeover</td>
</tr>
<tr>
<td>Provides robust, portable and transferable equipment</td>
<td>Supports globalization/regional/national production</td>
</tr>
<tr>
<td>Supports integration of up- and downstream processes</td>
<td>Permits enterprise schedule and control/closed system</td>
</tr>
<tr>
<td>Process development performed at manufacturing scale</td>
<td>Faster and more robust PD/lease of tech transfer</td>
</tr>
<tr>
<td>Reduces operator activity, decisions and FTE demand</td>
<td>Reduces staffing requirement and operational errors</td>
</tr>
<tr>
<td>Reduces equipment to be cleaned; services requirement</td>
<td>Supports sustainability/green initiatives; single-use</td>
</tr>
<tr>
<td>Operational ‘steady-state’; constant process loads</td>
<td>Heightens efficiency; supports continuous quality verification, continuous process verification and real-time release goals</td>
</tr>
<tr>
<td>Supports many product types, mass and stability demands</td>
<td>Supports enzymes, mAbs, vaccines and orphan drugs</td>
</tr>
<tr>
<td>Operating parameters fixed to one (optimized) range</td>
<td>Heightens processing parameter consistency/control</td>
</tr>
<tr>
<td>Reduced control actions, PLC activity and stress</td>
<td>Reduces process variance; product loss/reprocessing</td>
</tr>
<tr>
<td>Lowers process reaction and molecule residency times</td>
<td>Reduces postsecretion modification/degradation</td>
</tr>
<tr>
<td>Integrated, streamlined process stream w/fewer steps</td>
<td>Reduces intermediate storage, handling and QA risk</td>
</tr>
<tr>
<td>Reduces nonvalue added equipment and hold steps</td>
<td>Reduces handling errors as well as CPA and COG</td>
</tr>
<tr>
<td>Reduces development times and tech transfer steps</td>
<td>Reduces process development costs/risks/timelines</td>
</tr>
<tr>
<td>Increases process efficiency and process capability</td>
<td>Reduces materials/operating costs; risk and loss</td>
</tr>
<tr>
<td>Constantly operates at peak molecular efficiency</td>
<td>Raises materials and equipment utilization rates</td>
</tr>
<tr>
<td>Reduces capital expense/build costs/service demands</td>
<td>Increases profitability and portfolio possibilities</td>
</tr>
<tr>
<td>Supports a standardized, multiple-product ‘platform’</td>
<td>Increases overall equipment and facility utilization</td>
</tr>
</tbody>
</table>

Table 1. Values in continuous processing for pharmaceutical manufacturing.

COG: Cost of goods; CPA: Cost per action; EMA: European medicines agency; FTE: Full-time equivalent; mAb: Monoclonal antibody; PAT: Process analytical technologies; PLC: Programmable logic controller; QA: Quality assurance; QbD: Quality by design.
not be feasible for all products and processes, many implementations can provide a ‘platform’ approach in which a single process accommodates more than one product. CB nearly always shortens the process stream and its duration, reduces downtime and greatly reduces (manual) handling and storage of intermediates.

Table 2. Single-use perfusion-type culture at biomanufacturing scale.

<table>
<thead>
<tr>
<th>SU perfusion bioreactors</th>
<th>A: commercial examples</th>
<th>SU/hybrid PC application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed packed bed</td>
<td>CellDream™ (CerCell, Holte, Denmark), Celligen FibraCel (NBS, New Brunswick Scientific, Eppendorf, CT, USA), iCELLis (Pall, NY, USA) FB/FBS (Bioreactor Sciences, CA, USA)</td>
<td>SU fully controlled PC; adherent and some suspension culture</td>
</tr>
<tr>
<td>Hollow fiber</td>
<td>LSBR (FiberCell Systems, MD, USA), BiovaxID (Biovest International, MN, USA)</td>
<td>SU fully controlled PC; suspension and adherent culture</td>
</tr>
<tr>
<td>Moving packed bed</td>
<td>MBS (Bioreactor Sciences)</td>
<td>SU fully controlled PC; suspension and some adherent culture</td>
</tr>
<tr>
<td>Roller bottle</td>
<td>RollerCell 40 (CELLON SA, Luxembourg)</td>
<td>SU PC closed media exchange with some CPP control; adherent culture</td>
</tr>
<tr>
<td>Wave-action based</td>
<td>BIOSTAT RM (Sartorius Stedim, Aubagne, France), WAVE Xuri (GE Healthcare, MA, USA)</td>
<td>SU PC suspension and adherent (e.g., microcarrier) culture</td>
</tr>
<tr>
<td>SU perfusion capable</td>
<td>B: commercial examples</td>
<td>SU/hybrid PC application</td>
</tr>
<tr>
<td>Stacked array flask</td>
<td>RepliCell (Vericel, MA, USA), Xpansion (Pall, NY, USA), Cell Factory/ACFM (Thermo Scientific, MA, USA), HYPERStack (Corning, MO, USA)</td>
<td>SU PC closed media exchange with some CPP control; adherent culture</td>
</tr>
<tr>
<td>Stirred tank</td>
<td>Air-wheel (PBS Biotech, CA, USA), Integrity PadReactor (Pall), Mobius CellReady (EMD Millipore, MA, USA), SmartSystems (Finesse Solutions), S.U.B (Thermo Scientific), Xcellerex XDR (GE Healthcare)</td>
<td>SU PC potential when combined with perfusion-enabling technology; suspension and adherent (e.g., microcarrier) culture</td>
</tr>
<tr>
<td>Wave-action based</td>
<td>AppliFlex (Applikon, IN, USA), SmartRocker (Finesse Solutions), XRS (Pall), WAVE (GE Healthcare)</td>
<td>SU PC with perfusion-enabling technology Suspension and adherent (e.g., microcarrier) culture</td>
</tr>
<tr>
<td>Perfusion enabling tech</td>
<td>C: commercial examples</td>
<td>SU/hybrid PC application</td>
</tr>
<tr>
<td>Centrifugal media exchange</td>
<td>Centritech (Carr), kSep (KBI Biopharma)</td>
<td>SU PC when combined with certain bioreactors; suspension or adherent (e.g., microcarrier) culture</td>
</tr>
<tr>
<td>Hydrocyclone media exchange</td>
<td>Hydrocyclone (Sartorius)</td>
<td>SU PC when combined with certain bioreactors; suspension culture</td>
</tr>
<tr>
<td>Hollow fiber media exchange</td>
<td>ATF System (Refine Technology), CFP Cartridge (GE Healthcare), KrosFlo (Spectrum), MabTech (Parker Domnick Hunter), Quantum (Terumo BCT)</td>
<td>SU PC when combined with certain bioreactors; suspension or adherent (e.g., microcarrier) culture</td>
</tr>
<tr>
<td>Sonic wave media exchange</td>
<td>AWS (FloDesign Sonics, MA, USA), BioSep (Applikon), CYTOPERF (APicells, MA, USA)</td>
<td>SU or hybrid PC when combined with certain bioreactors; suspension culture</td>
</tr>
<tr>
<td>Spin filter media exchange</td>
<td>Spinfilter P (Sartorius Stedim)</td>
<td>SU PC when combined with certain bioreactors; suspension or adherent (e.g., microcarrier) culture</td>
</tr>
</tbody>
</table>

ATF: Alternating tangential flow; PC: Perfusion culture; SU: Single use.
CB extends such capabilities to advancing continuous quality verification, continuous process verification and real-time release enabling initiatives. Although CB will not be feasible for all products and processes, many implementations can provide a ‘platform’ approach in which a single process accommodates more than one product. CB nearly always shortens the process stream and its duration, reduces downtime and greatly reduces (manual) handling and storage of intermediates.

CB demands increased near real-time process monitoring in support of the comprehensive control procedures required to maintain the ‘steady’ states involved. In support of this, SU flowpath systems now exist from such sponsors as Nova, Flonamics, Bend, Groton and Bayer for the automated withdrawal and processing of whole or even cell and microcarrier-free samples for online, multianalyte or multivariate monitoring [34–36].

However, concerns have been expressed regarding the implementation of such a disruptive technology. They include performance reliability (incidence of failure), validation complexity, integrated process control requirement, economic justification and lot definition. But for many processes, such previous limitations – or their perception – are being alleviated by specific advances in CB processing technology or by Operational Excellence (OpEx)-driven technological advances described above. There are, nevertheless, such ongoing challenges as:

- Equipment’s validation for extended operation;
- Mass transfer at intensified perfusion densities;
- Global/enterprise event/flow process control;
- Concerns for cell-line stability in extended runs.

The latter point 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 additional generation number, this fact needs to be validated for each clone.

While the demand for many services and consumables are reduced in CB, one that is not is cell culture media. Large quantities of media are required to be supplied continuously to the perfusion bioreactor. However, continuous, automated inline culture medium and buffer dilution, conditioning and dispensing 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 ICE (GE Healthcare, MA, USA) is a currently popular system addressing these challenges.

**Benefits of SU continuous biomanufacturing**

Operationally, CB’s time-effected product mass accumulation allows for a continuously variable manufacturing rate and inherently promotes ‘scaling-out’ or ‘numbering-up’ to increase maximum capacity, which also greatly facilitates process standardization and accommodation of SU systems. SU CB contributes to overall process flexibility in that its equipment tends to be easy to clean, inspect and maintain and ease of product changeover because it tends to be more modular, reconfigurable and transportable than traditional stainless equipment [37].

CB contributes to reduced process development times in a number of ways. These complement SU-specific efficiencies provided by, for example, elimination of the requirement to develop services supplying cleaning and steaming steps. Other SU advantages here are its accommodation of an open architecture approach as well as a number of hybrid designs. Such design flexibility includes equipment combinations of between reusable and disposable systems, divergent suppliers and locations, or of particular equipment styles. As applied to CB we can see the many flexibilities of SU provide a manufacturing platform of exceptional efficiency, adaptability and operational ease [38]. Advances in the engineering of SU-transfer tubing/systems, distribution manifolds, container porting and fluid impulsion also promote creativity in process design. This is of particular value in designing a process with such demands as an entirely new flow path, process monitoring and control, or lot designations – such as for CB. Creative development in process flow and flexible configurations in perfusion culture and downstream activities is required in CB for many reasons, including:

- Commercially available CB solutions are still in development;
- Procedures for optimal production have not been discovered;
- Refined procedures that do exist are generally not published;
- New development in such CB support as intensified perfusion;

**Key term**

**Perfusion culture:** The culture of cells through their isolation and the exchange/renewal of either culture medium metabolites and gasses or the whole culture medium fluid itself. Can be classified by the type of cell (e.g., suspension/adherent), type of exchange (e.g., metabolite/whole medium), the cell-isolation process (e.g., filtration/settling) or the culture mode (e.g., stirred tank/packed bed).
• New understandings in perfusion culture metabolic demands;

• Developments in solutions for perfusion mass-transfer needs.

As CB processes have greatly simplified production trains, they inherently facilitate application of closed processing approaches to individual operations and even processes. Such features as the modularity and integral gamma irradiation sterility of SU materials and systems combined with the simplicity and sustained operation of CB promise the appearance of platforms of reduced processing time and increased operational ease [28].

Beyond CBs higher inherent operational efficiency, coincident benefits from the improvements in multiply-recombinant producer clone generation and intensified feeding strategies are determining that a reduced volume of (and less expensive) culture medium is demanded and more concentrated perfusion bulk intermediate product is being generated. Smaller containers and storage suites are now required for both surge protection and (hybrid process) product intermediate containment – both of which play right into the efficient application of SU systems and technologies. These features all contribute to promoting the common goals of reducing capital expenditures, minimizing project timelines and increasing operational flexibility – while minimizing operational costs (Table 3). But the advantages here go even beyond this. Employment of SU equipment in a continuous bioprocess flow promotes the design of closed and highly integrated operations [39]. This is enabling the growing ‘Factory-of-the-Future’ initiative including the manufacturing even divergent product types in grayscale ‘ballroom’ suites of reduced classification introduced in the section ‘Single-use systems’ [40].

Controlled nonclassified is a designation often used in noncritical areas in GMP manufacturing facilities. In regulated closed-system processing, the status of the manufacturing suite becomes secondary to the integrity of the closed systems. Biomanufacturing processes employing closed operations in an environment of reduced classification is a highly desirable goal actively pursued in many venues [41]. For example, there are ongoing EU GMP Guideline/ICH Q3C (R4) issues concerning new toxicological models and more science-based dispositions toward contamination, cross-contamination and multiproduct manufacturing. This has not only often been invoked in regard to closed manufacturing, but also has implications in SU continuous biomanufacturing. Its resolution should clarify synergies and paths forward in creative processing modes, process flow as well as facility design and classification.

Continuous manufacturing processes, due to the integral nature of their contiguous operations, inherently lend themselves to such closed operation. Surprisingly, there is ongoing work regarding the precise nature of a ‘closed process’ within biomanufacturing for chemistry manufacturing and controls (CMC) purposes. For example, the ISPE currently advises within its definition of the term ‘It is the manufacturer’s responsibility to define and prove closure for a process step’ [42]. Nevertheless, by exploiting these system feature correlations and designing a functionally closed manufacturing flow path, a SU CB facility can be envisioned with combined work areas not requiring class-

### Table 3. A Biopharm Services BioSolve Process model of continuous versus fed batch monoclonal antibody processes in stainless steel versus single-use based facilities.

<table>
<thead>
<tr>
<th>Configuration</th>
<th>100 kg/year</th>
<th>500 kg/year</th>
<th>200 kg/year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Capex (US$10)</td>
<td>COG (US$/g)</td>
<td>Capex (US$10)</td>
</tr>
<tr>
<td>Stainless</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch</td>
<td>79</td>
<td>318</td>
<td>131</td>
</tr>
<tr>
<td>Hybrid</td>
<td>81</td>
<td>306</td>
<td>116</td>
</tr>
<tr>
<td>Continuous</td>
<td>59</td>
<td>217</td>
<td>79</td>
</tr>
<tr>
<td>Single use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch</td>
<td>21</td>
<td>163</td>
<td>47</td>
</tr>
<tr>
<td>Hybrid</td>
<td>24</td>
<td>147</td>
<td>45</td>
</tr>
<tr>
<td>Continuous</td>
<td>32</td>
<td>144</td>
<td>48</td>
</tr>
</tbody>
</table>

Capex: Capital expenditure; COG: Cost of goods.
Reproduced with permission from Biopharm Services (Beaconsfield, UK), 2014.
Single-use perfusion bioreactors support continuous biomanufacturing

Review

Such a facility offers many potential benefits, including a reduction in:

- Construction costs;
- Start-up schedule extent;
- Utilities (clean water and steam);
- Manufacturing suite area and barriers;
- Suite classification (and maintenance costs);
- Manufacturing suite operation steps and costs;
- Heating, ventilation and air-conditioning (HVAC; and related plant and quality maintenance).

What has been presented so far relates to existing products and manufacturing platforms. Recent trends in other manufacturing imperatives also synergize with many of the SU and CB coincident values and features described here. These new manufacturing goals and considerations include the:

- Need for decentralized, local sourcing/production;
- Lower mass-demand ‘next-gen’ products;
- Demand for reduced development times;
- SU real-time product quality monitoring;
- Globalization of production competition;
- Trend toward contract manufacturing;
- Development of less-stable products;
- Explosion in biosimilar development;
- Price-sensitive/controlled markets;
- Growth of multiproduct facilities;
- Demand for process flexibility;
- Need for pandemic response;
- Increased volumetric yield.

CB promotes increased profitability, beginning with reduced capital expense; invariably reducing equipment footprint and facility extent and its offering high equipment utilization rates [43]. This is a consequence of a CB batch being primarily determined by run time rather than reaction container size, thereby reducing the size of a bioreactor. But, there are a number of other features contributing to this (Table 1). CB can lessen the need for operator intervention (and therefore support personnel) as well as reduce nonvalue-added operations because it simplifies a process and optimizes process flow. Also, by reducing such nonvalue-added steps as intermediate product hold and final product inventory, CB reduces the faculty and quality systems (QS) requirements for their storage. This completely harmonizes with SU technology and systems which themselves present reduced validation requirements, quality systems maintenance, controlled environment extent and operations personnel.

In summary, other than the usual hesitation regarding anything new, there really are a few real financial, engineering or regulatory concerns to preclude the serious consideration of SU CB in pharmaceutical manufacturing. Industry leaders see the design of closed, disposable, integrated and continuous biomanufacturing systems for biopharma on the near horizon.

Perfusion culture

Virtually the only CB-supporting upstream processes intensification in animal-cell-based bioproduction are variations large-scale continuous-flow cultures. These are most often perfused cultures operating in some type of chemo- or turbidostat [44,45]. In such a perfusion mode, cells are retained through a continuous or transient immobilization, isolation or concentration in some way to allow older culture medium (or metabolites) to be withdrawn and replaced by fresh medium (or metabolites). In the past, because of a number of inherent limitations, perfusion culture in biomanufacturing was primarily reserved for unstable molecules. However, recent developments in supportive technologies are supporting its application more generally. For example, serum-free and defined media formulations and supplements have evolved to sustain high cell densities (50–100 × 10⁶ cells/ml) in perfusion systems, providing unprecedented productivity. The number of perfusion(-like) systems appearing range from novel innovations in smaller working volume (such as rotary or rotating wall culture) to variations on large-scale TFF-based systems where cultures are driven to unusually high densities [46]. Principal values afforded by perfusion, semiperfusion, intensified perfusion or perfusion-like cultures include:

- Products significantly reduced reactor residency duration;
- Potential for (practical) ‘steady states’ during production;
- Dramatic and sustained growth in volumetric productivity.

It should be mentioned that beyond equipment and process flow developments, there are cell-biol-
ogy-based technologies contributing to the establishment of novel perfusion approaches. Alterations in the nature or timing of the culture’s nutritional environment can result in dramatically improved cell performance or product accumulation. Introduction of recombinant genes can alter the transcriptional, translational or other metabolic behavior of cells in perfusion culture. Flow systems operating in (quasi-) steady state conditions often diverge from true equilibrium of state variables. This, as well as innovation in bioreactor engineering, have caused some ambiguity in application of the terminology describing the perfusion (-like) bioreactors in use today (Table 4).

Quite a number of creative equipment and process solutions to the earlier challenges encountered (especially in scale-up) have been engineered [47–49]. They include many flavours of fluidized bed, centrifugal concentrators, gravity-based (conical and inclined ramp) settlers, hydrocyclone, packed bed, spin filters, ultrasonic resonators/filters, as well as crossflow membrane and diverse (internal and external) hollow fiber-based systems. Such activity as the Bolt-on Bioreactor initiative [50] illustrates the extremely dynamic nature of the industry. Perfusion culture solutions now exist for a variety of secreted protein biologics, vaccine and cell therapy applications and those employed for many years well described and reviewed [51,52]. Pertinent here are those systems engineered in single-use components at the manufacturing scale (Table 2).

It is important to consider that continuous processing, including perfusion culture, can place increased or unique pressures upon manufacturing systems and especially SU systems. 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’. CBs introduction has determined a re-examination of a few related concepts in this regard (Table 5). 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. One must carefully examine the unique stresses that the extended operation of continuous processes place upon equipment, as well as the increased mass transfer demands an intensified perfusion mode invokes. While some perfusion technologies could theoretically be operated indefinitely, such practical considerations generally limit the animal cell culture durations currently addressed to between 20 and 60 days.

Table 4. Concepts in upstream continuous processing in techniques: some with overlapping features.

<table>
<thead>
<tr>
<th>System</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialysis</td>
<td>Primary/secondary metabolites exchanged across a membrane</td>
</tr>
<tr>
<td>Extraction</td>
<td>A two-phase system which lowers some secondary metabolites</td>
</tr>
<tr>
<td>Perfusion</td>
<td>Media continuously exchanged (e.g., gravity/filter/centrifuge)</td>
</tr>
<tr>
<td>Enhanced perfusion</td>
<td>Media continuously exchanged with cells greatly concentrated</td>
</tr>
<tr>
<td>Perfusion-like</td>
<td>Any of the growing ‘not-quite’ or ‘semi’ perfusion approaches</td>
</tr>
<tr>
<td>Steady state</td>
<td>Equilibrium-like, but establish with balanced inputs and outputs</td>
</tr>
<tr>
<td>Internal concentration</td>
<td>Integral cell concentration by, for example, inclined ramp or hydrocyclone</td>
</tr>
<tr>
<td>Internal filtration</td>
<td>Media exchanged and cells retained through a (static/spin) filter</td>
</tr>
<tr>
<td>External filtration</td>
<td>Media exchanged through some external (virtual) filtration unit</td>
</tr>
<tr>
<td>Hollow fiber perfusion</td>
<td>Media changed and cells retained within a hollow fiber cartridge</td>
</tr>
<tr>
<td>Continuous</td>
<td>Prolonged feeding/harvest control maintaining a ‘steady state’</td>
</tr>
<tr>
<td>Chemostat</td>
<td>A steady state-type where culture expansion equals dilution rate</td>
</tr>
<tr>
<td>Repeated</td>
<td>A fraction of the biomass provides seed for the next culture cycle</td>
</tr>
<tr>
<td>Attached continuous</td>
<td>2D stacked array multiplate or stirred suspended microcarrier 3D scaffold fixed or moving packed-bed perfused bioreactors</td>
</tr>
</tbody>
</table>
Single-use perfusion bioreactors

SU technologies provide specific and enabling features in continuous biomanufacturing implementations [53]. SU features that particularly complement CB range from low initial investment costs, to contributing to reduced time-to-market, to promotion of heightened process control [54, 55]. Much of the SU equipment offerings for batch bioproduction have the same or related application in CB systems: from simple equipment as tubings and genderless connectors to such complex assemblies as bioprocess containers for the cryopreservation of large working stock aliquots. Most commercially available large-scale SU bioreactors are capable of accommodating perfusion culture of some type, when fitted with appropriate ancillary equipment (Figure 1) [56, 57]. Some, such as packed-bed reactors, inherently lend themselves to perfusion operation without modification.

A variety of fluids are supplied to, and collected from, perfusion culture systems throughout operation – and number of SU products exist to support the production and distribution of such reactor charge, feed, sampling and recycled fluids in perfusion applications. Large-volume activities such as culture media formulation are facilitated by, for example, SU mixers, manifolds, pumps, valves and aseptic connection systems [58].

The synergy of employing a process that requires clean-in-place/steam-in-place (CIP/SIP) only once each 1–3 months, with a format the does not require the service at all, is obvious. In addition to promoting such advanced process development and operational goals, for many of the values described above both SU systems and CB require a significantly simplified process control strategy. They present heightened integrated processing potential, with fewer steps or operations, and thus can provide reduced process variability, human machine interface (HMI) activities and provide (ultimately) a more integral comprehensive (even statistical) system control. Such correlative features result in dramatically increased overall facility efficiencies, further reducing both capital and operational expenses.

The heart of upstream CB is the bioreactor. At the research scale there have been SU hollow fiber perfusion bioreactors available from a variety of vendors for over 40 years. At even manufacturing scales there have been steel construction perfusion bioreactors in use for over 20 years. However, only recently have SU manufacturing-scale perfusion-capable equipment appeared [59] and their coordinated implementation in production settings is just now beginning [60]. One key enabler of the application of SU reactors to perfusion and intensified perfusion is the continued development of sparging apparatus and techniques – and previous mass-transfer limitations have for the most part been overcome.

In fact, SU systems are available for most any process format (e.g., microcarriers and suspension), platform (e.g., cell line, vectors and culture media), mode (e.g., dialysis or intensified perfusion) or scale (e.g., through rapid, inexpensive horizontal scale-out). ‘Future-proofing,’ or sup-

### Table 5. Operational qualification concepts.

<table>
<thead>
<tr>
<th>Qualification concept</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>Reusable</td>
<td>Equipment or material intended for use for an indefinite number of times: especially in different production cycles or batches, and after salvaging or preparation by special treatment or processing</td>
</tr>
<tr>
<td>Multi- or limited-use</td>
<td>Equipment or material intended for use in a process for a limited number of cycles: determined by validated procedure or subsequent testing</td>
</tr>
<tr>
<td>Single-use</td>
<td>Equipment or material intended for use in a process for one cycle and then retired from use</td>
</tr>
<tr>
<td>Hybrid</td>
<td>Equipment, material or operation composed of both reusable and single-use components</td>
</tr>
<tr>
<td>Disposable</td>
<td>Equipment or material intended for use either for one time or for use in a process for a limited number of times, and then retired as waste</td>
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Continuous biomanufacturing modifies operational qualification concepts. Continuous biomanufacturing can dramatically increase the duration and throughput volumes involved in each ‘use’. Review of the (pre)validation requirements advised.

**Key terms**

- **Single-use perfusion bioreactor**: Single-use bioreactors supporting some type of perfusion culture. In biomanufacturing, it is implied that they are of manufacturing scale and commercially available as either an integral system or as complementary systems components of acceptable assembly.

- **Hollow fiber perfusion bioreactor**: Supports direct culture of suspension/adherent cells employing integral microporous hollow fibers. Can be further classified into intrafiber or extraliber culture.

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porting the sustainability of a new CB process in the face of product life cycle or emerging technology imperatives, is enabled by SU’s low initial facility, service and equipment costs, undedicated manufacturing suite requirement and ease of process train reconfiguration.

One can see why processes which are CB as well as SU are being adopted for the production of many existing protein biologicals and vaccines. But, there are many developing cell-culture formats and expression systems serving a growing number of entirely new entities and product types. SU CB approaches serve not only today’s demands, but next-generation viral and protein biological products (Table 6) as well as therapeutic platforms of various bulk mass demand including:

- Bioartificial organs;
- Therapeutic (pre)stem cells;
- Gene therapy nucleic acid vectors;
- Expanded differentiated organ cell mass.

For example, we know there are over 20 antibody conjugates now in development. As the preconjugate antibodies are generally produced in Chinese hamster ovary/nonsecreting zero (CHO/NS0)-type suspension transfectoma, we see them as amenable to SU, perfusion approaches. Interestingly, we also see SU and CB technologies now being applied in even novel bacterial vaccine production [61].

Environmental objectives are supported by the fact that CB operations can reduce the:

- Amount of equipment to be processed in cleaned or steam sterilization;
- Process steps, footprint, service requirements and energy consumption;
- Numbers of support personnel (and their commuting to work) required.

Advanced means of environmental impact assessment and reporting for each of CB manufacturing approaches and SU materials and have been well reviewed [62], and it is of note that none have been reported in conflict with each other [63].

**Mechanically agitated suspension bioreactors**

Mechanically agitated (often stirred-tank) bioreactors, containing either suspension or anchorage-dependent cultures on a support matrix feature well-understood geometries and performance characteristics resulting in adaptable and robust operation. Here, suspension or matrix-attached cultures are agitated through a number of mechanical mechanisms, from marine impellers to paddles to airwheels. The stirred tank bioreactor (STR) is the most popular of the suspension systems for such reasons as it is simple to operate, easy to scale up and is well understood. One advantage here is that suspension cell systems do not depend on constrained surface area. This allows significant culture intensification and operational cell densities obtainable quite variable and amenable to intensified perfusion mode cultures of over $10^8$ cells/ml. For attached cultures, several types of commercially available microcarriers include the popular dextran-based microcarriers from GE Healthcare and a variety of SoloHill microcarriers from Pall Life Sciences (NY, USA) [64,65]. STRs are easily converted to culture systems accommodating continuous processes through the addition of ancillary perfusion enabling devices (Figure 2). Examples of such bioreactors and related ancillary filtering apparatus’ are listed in Table 2B 

**Fixed/floating filter bioreactors**

Presterilized SU wave-action (or rocking) bioreactors provide another perfusion-capable solution that is scalable to 1000 l (Table 2A & B). Here, disposable bags with no integral impeller are inflated and rocked to provide oxygen transfer and mixing [66]. The WAVE Bioreactor™ from GE Healthcare is available with integral perfusion culture capability (Table 2B) [67]. Many will support an external transient cell immobilization-enabled perfusion. These bioreactors provide such general service as air inlet and outlet filters, needleless sampling ports, dissolved oxygen probes insertion

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**Key terms**

**Mechanically agitated suspension bioreactor:** Bioreactors supporting the culture of free or particulate-bound cells (e.g., microcarriers) through continual mixing (e.g., by an impeller or paddle).

**Filter bioreactor:** Supports mechanically agitated intensified batch or continuous perfusion culture through the retention of cells/microcarriers by an external or internal (e.g., fixed, floating or spinning) porous membrane, fiber or screen. Clogging of screen is greatest limitation and is ameliorated by various technologies.
ports and fill/harvest ports – all conforming to USP Class VI specifications. They can be customized with optional fittings such as aseptic connectors, dip tubes, an optical pH sensor embedded in the bag, screw cap ports, temperature ports, perfusion filters and special tubing ports. Some employ a unique floating filter that takes advantage of the wave motion to keep the filter surface from clogging and yield a simple, disposable perfusion bioreactor suitable for biotechnology and medical applications.

**Packed-bed bioreactors**

**Packed-bed (PB) bioreactors** are a type of entrapment culture providing continuous culture enabling upstream process. They are capable of providing a variety of cell lines for long periods of time while providing some rare and valuable performance features. One example is the extremely low shear established due to the immobilization of cells within their integral macroporous matrices. This concept has been applied for decades in a number of implementations, many of which failed due to inherent design faults. The newest configurations have specifically addressed these issues, as well as introducing such improvements as single-use flowpaths. PB reactors are currently employed in a number of diverse manufacturing, research and therapeutic applications.

The successful implementation of commercial PB reactors in support of large-scale recombinant biologic and vaccine manufacturing has been accomplished [68]. There are quite a few commercialized PB reactors on the market and examples of those with manufacturing-scale SU flow path perfusion capability are listed in Table 2A.

Packed bed type systems can be further classified into two types: fixed bed and moving bed. Most PB bioreactors for cell culture are fixed bed systems, but the FB/FBS Bioreactor (Bioreactor Sciences, CA, USA) promises a single-use solution with a moving bed, and this provides some rather unique flexibility and functionality. PB bioreactors commonly use similar medical-grade, macroporous nonwoven polyester fiber carriers that differ principally in surface treatment, configuration and structure. The fiber carrier’s matrix presents a plurality of interconnected, open pores with essentially no closed pores. In some implementations suspension cultured cells are introduced to the bioreactor during seeding and continue as a suspension culture within the matrix cavities. Here, they are not specifically adherent to the porous matrix surfaces, but kept in agglomerated state within the matrix boundaries in combination of semiahesive and/or suspension state. The use of such carriers also provides for easy separation of the ambient media from the attached cells, supporting convenient media replacement, perfusion culture or final harvest.

Mass transfer, and therefore kLa values, for packed bed reactors can be different from those for typical stirred tank reactors for such reasons as the absence of cells in the medium in circulation for gas exchange. Values can also differ greatly between the various packed bed reactor styles because of distinctions in the packed bed versus overall working volumes, recirculation rates and overall oxygen uptake rates. For example, cells in the iCellis (Pall Corporation, NY, USA), and Celligen BLU Fibra-Cell (Eppendorf, CT, USA) reactor surfaces are continually submerged in media, relying upon aeration mechanisms to supply oxygen. Diffusive mass transfer in the Celligen depends on agitation as well as sparging, while the iCellis relies on its defined waterfall surface area. The TideCell and FB/FBS use the principle found in roller bottles, exposing cells directly to air for oxygen transfer. Employing this direct-to-air principle simplifies production scale-up and eliminates the foaming and shear issues commonly encountered with other approaches to aeration.

The TideCell uses bidirectional flow and multiple internal tubes to mitigate some scaling challenges. Both the iCellis and the BioCell MB use shallow beds and unconstrained flow rate to resolve these scale-up challenges.

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**Figure 2. Perfusion-based bioproduction accomplished in a Xcellerex XDR single-use bioreactor from GE Healthcare (MA, USA) configured with a ATF6 System from Refine Technology (RepliGen, MA, USA).** A single-use version of the ATF6 is appearing which will support a fully single-use flow path.
issues. Some present an additional concern of using less than twice the volume of medium around their bed, which determine a requirement or frequent/continual medium replacement/feeding to maintain nutrient and oxygen supply. The medium volume for the TideCell is variable, supporting over 5x the bed volume and creating a robust environment for the culture.

CerCell provides configurable SUBs that support batch, fed-batch and perfusion modes [69]. Their patented CellCore provides a magnetically impelled specialized dynamic flow through a parallel array of cavities. The cells are maintained in a proprietary porous matrix scaffolding of selectable properties and variable diameter. Properties of the scaffolding, such as porosity and pore size, can be specified to fit individual culture formats with some applications supporting densities beyond 100 million cells/ml. This fixed packed-bed system eliminates gradients in primary and secondary metabolite and supports the culture of both adherent and suspension cell lines. A popular implementation currently available provides the productivity equivalent of a 1000 (1 m³) stirred-tank SUB per day. The CellCore is becoming available in growing number of working volumes such as in their CellTank. Part of the standard package is a purpose-designed process control system with sensors for such parameters as glucose and cell-mass, which allow proportional integral derivative (PID) regulated feeding, perfusion flow and cell-mass bleeding, among others. This provides for continuous, industrial up- and downstream processing in one single-use unit assembled from a repertoire of application-specific components.

**Hollow fiber perfusion bioreactors**

Hollow fiber perfusion bioreactors (HFPBs) represent one type of high-density immurement culture that supports continuous perfusion (Table 2A). While not a stirred-tank suspension-mode type, they are not truly packed-bed reactors either. In the most common implementation mammalian cells are seeded inside the cartridge body, but on the exterior of the hollow fibers in what is known as the extracapillary space (ECS). In this configuration, culture medium is pumped through the interior of the hollow fibers allowing nutrients and secondary metabolites to diffuse through the fiber walls in each direction. Medium flowing from the cartridge can be subsequently oxygenated and reintroduced to the loop, or it can be collected for processing of product as fresh medium is introduced. The basic features of a HFPB system include:

- Extremely high culture binding surface-to-volume ratios;
- Immobilization of cells at very-high (biomimetic) density;
- Culture on a porous matrix supporting prolonged culture;
- Selectable fiber porosity providing segregation of solutes.

The high-density culture in such controlled hydrodynamic conditions as an HFPB can provide a micro-environment of directional flow, establishing a gentle interstitial gradient within the cell mass for autocrine stimulation, cell alignment, and desirable cell–cell or cell–surface interactions. Because an HFPB cell culture (on the ECS side of the fibers) can exist at concentrations greater than or equal to 100x that of standard suspension cultures, it was discovered early on that continuous culture in less animal serum may be more easily established and provide several benefits over classical cultures modes [16,70–71]. To support this, a serum replacement (CDM-HD, FiberCell Systems) was designed to take advantage of the unique cell culture conditions found within a hollow fiber bioreactor. Besides being optimized for continuous culture, this chemically defined serum-free supplement is surfactant, protein and animal-component free. HFPB systems allow for the long term support of divergent cell types in coculture at even extreme ratios [16]. The FiberCell Duet and the new large-scale LS-HFBR from FiberCell Systems have demonstrated performance in providing single-use flowpath perfusion culture with a diverse array of adherent or even suspension cell lines [16].

**SU accessories supporting perfusion culture**

**Hollow fiber medium exchange**

A number of external hollow fiber-based perfusion devices exist (Table 2C). Repligen Corporation is launching a SU version of their popular ATF external cell separation system using alternating tangential flow through hollow fibers of a filter module. Benefits of the ATF System include nearly linear scale-up (from >1 to <2000 l), simplicity of operation and validation, plus a choice of filter materials and pore sizes. Traditional TFF in comparison can become clogged in time from cell agnates in the pores or accretion of debris across the membrane surface. In a one-directional flow system, aggregates lodging in the hollow fiber (HF) will diminish filtration capacity by the degree of blockage. The combination of a reversible flow through the filter with high and low pressure cycles results not only in an efficient tangential flow effect, but also in significant transmembrane fluxes to prevent fouling even at cell concentrations in the
order of $200 \times 10^6$ cells/ml. Many CMO’s as well as ‘instigator’ or premiere biopharmaceutical sponsors are currently employing the alternating tangential flow (ATF) with SUBs in hybrid enhanced perfusion and continuous culture applications. When employed with SUBs, the single-use ATF just now being introduced will provide for an entirely SU flowpath enhanced perfusion system [72].

Spectrum Laboratories (CA, USA) KrosFlo Perfusion System is an entirely single-use flowpath means of transient immurement, enabling external HF based perfusion culture. This cultured cell and microcarrier filtration accessory product comes sterile and ready-to-use, providing such benefits as a dramatically reduced set-up time. It employs SU low-shear levitating pumps, reducing impact on cell viability. The manufacturer advertises up to $400 \times 10^6$ cells/ml in a noninvasive flowpath with inline pressure sensors and cartridges containing polyethersulfone or polyethersulfone hollow fibers. It is scalable, with commercial implementations from 2 to 2000 l in application with either reusable or SUBs. The automated KrosFlo Perfusion System (aKPS) is a more comprehensive solution featuring feedback control loops, control of the recirculation pumps, backpressure valves, permeate pump and high-pressure circuits, touchscreen HMI, permeate scale and distributed control system (DCS) hardware [73].

Continuous-flow centrifugation

High capacity continuous flow centrifuges are available with a wide variety of rotor designs capable of dynamic loading and unloading while the rotor is spinning. We focus here on those enabling a SU perfusion mode of cell culture (Table 2C). Each is economical to use, as even the disposable components have robust longevity within a run with no mid-run degradations such as filter ageing or clogging. Since the parameters that control the separation are g-force, time and flow rate, such things as filter capacity or surface areas are not a concern. Cell separation takes place in a presterilized module constructed from Class-VI pharmaceutical grade materials. Once the module is installed and tubing connected using an aseptic technique or sterile welding, the systems are virtually closed, providing a high degree of aseptic or sterile reliability without the need for CIP or SIP (Figure 3). While different products employ distinct separation principals, essentially the cell suspension is fed into an inlet at one end of the module and the cells are centrifugally concentrated or separated from the media. Low-shear isolation of mammalian and insect cells is possible and minimal reduction in viability of recovered cells achievable. Clarified supernatant is discharged from one outlet and cell concentrate from yet another outlet, possibly in a distinct periodic cycle. These devices can support both perfusion culture and bioreactor harvest operations.

Three limitations for centrifugal-based perfusion have been noted:

- Process development: there exist no true scale-down models;
- Output constraints: they do not generate some desired cell concentrations;
- Input constraints: faster, slower or smaller feed volumes can be problematic.

CARR Centritech’s CELL8 (Oakville, Canada) from utilizes a gamma irradiation sterilized single-use module. The modules contain no rotating seals and are readily connected to a SUB to establish either SU perfusion culture of clarified harvest. The basics of perfusion operation are that the cell suspension is gently pumped to the module and the cells settle to the lower outer radius via low G-force while debris and supernatant are continuously discharged. The CELL8 has a demonstrated track record in the perfusion of cell cultures as well as the differential harvest of supernatant, cells, dead cells and debris. It can operate at 36–320× g and support bioreactor volumes of up to 3000 l working at t flow rates of 6–120 l/h [74].

**Key term**

**Centrifugation-based perfusion:** Supports mechanically agitated intensified batch or continuous culture, without such invasive components as membranes, through the retention of cells/microcarriers by continuous flow centrifugation of various rotor design. They can provide consistent performance with no mid-run degradations such as filter ageing or clogging.

---

**Figure 3.** The single-use module from a CARR Centritech (Oakville, Canada) closed, continuous bioprocessing capable centrifuge.
The kSep6000S from kSep Systems also provides automated class VI single-use flowpath perfusion support, yet through an entirely different technology [7]. In this GMP closed system unit, a fluid flow force counteracts the centrifugal force and creates a fluidized bed of cells that remain in suspension throughout the process. There is a continuous operation with the chamber emptying by reversal of flow, and the chamber never stops rotating. Through the equilibrium of pumped media flow and centrifugal forces, the main chamber retains such particulates as cells or microcarriers as a concentrated fluidized bed within the gently flowing media or buffer. Constant oxygen and nutrient supply in the 6 l (6 × 1000 ml) volume chamber is supported by an over 10 l/min flowrate with a total processing volume of from 100 to 6000 l. Flow rates ranging from one-half to over 10 l per hour and a 600–1200 billion cell capacity per cycle. This automated sequence available is advertised being useful in cell banking, manufacturing in cell-based therapy and a number of vaccine production processes.

**Acoustic wave separation**

Separation by ultrasound is a technique for the isolation of small particles from fluids without the need for invasive components such as membranes, or the moving components required by centrifuges or spin filters. It is a nonfouling and nonclogging retention device for perfusion applications. Through what are variously referred to as ultrasonic resonators, separators or filters, acoustic wave separation (AWS) has been essentially demonstrated for decades – but only recently successfully applied to practical large-scale animal cell separation in perfusion culture and reactor harvest applications (Table 2C).

Ultrasonic separators are comprised of two components: an ultrasonic controller generating an electric driving signal of defined power and frequency, and a chamber of a particular geometry where the driving signal is converted into an ultrasonic standing wave field that inhibits the dispersion of cells flowing through the chamber. An important aspect of the system is that (in distinction to some physical sieve systems) it produces virtually no shear or hydrodynamic stress on the cells whatsoever. In contrast to other cell-separation techniques, the acoustic energy constitutes a ‘virtual’ screen or mesh, and thus provides a superior noncontact, nonfouling, nonmoving means of cell separation. Product concentration has been reported to increase up to fivefold, allowing a practical reduction of the required bioreactor volume of up to 100-fold. One commercialized product, the AppliSens Biosep Acoustic Separator, has been optimized for the separation and retention of cells in perfusion-cell-culture processes is marketed by Applikon Biotechnology (CA, USA) [76].

The Wave D3TM separation technology developed and patented by FloDesign Sonics (MA, USA) is now of interest in reactor harvest clarification, but is being considered in bioreactor perfusion as well. Its performance was recently reviewed by Merrimack Pharmaceuticals in the context of the popular centrifugation and TFF approaches. In the report, this nonoptimized AWS process favorably compared with a full-scale DFF GMP process [77].

Another product, the Cytoperf from APIcells (MA, USA) is the first commercially available fully disposable AWS device addressing SU flowpath demands in high-density perfusion culture. As a nonfouling and nonclogging retention device this disposable acoustic

### Table 6. Single-use continuous biomanufacturing potential in next-gen products.

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<th>SU potential</th>
<th>CB potential</th>
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<tr>
<td>Biosimilars, biobetters, cocktails, synthetic light chain and bioconjugates, BSMAbs, polyclonals and Fc fusions</td>
<td>Yes – CB</td>
<td>Mostly yes</td>
</tr>
<tr>
<td>New protein biologics for cancer, osteoporosis, ophthalmic...; domain antibodies, dAbs, other next-generation fragments</td>
<td>Yes – CB/CF</td>
<td>Yes</td>
</tr>
<tr>
<td>Designed ankyrin repeat proteins (DARPins) Anticalins, adnectins and other alternate scaffolds</td>
<td>Yes – CF</td>
<td>Yes</td>
</tr>
<tr>
<td>Viral vectors, VLPs, Vravacines and conjugates</td>
<td>Yes – CB</td>
<td>Nonlytic</td>
</tr>
<tr>
<td>Dual-ligand peptides, ‘Bicycles’ and assemblies</td>
<td>Yes – CF/CM</td>
<td>Yes</td>
</tr>
</tbody>
</table>

BsMAb: Bispecific monoclonal antibody; CB: Continuous biomanufacturing; CF: Continuous fermentation; CM: Continuous manufacturing; dAb: Single-domain antibody; rVaccine: Recombinant vaccine; SU: Single use; VLP: Virus-like particle.

**Key term**

**Acoustic wave-based perfusion:** Supports mechanically agitated intensified batch or continuous culture through the retention of cells/microcarriers by ultrasound-based isolation of without the need of invasive moving components are variously referred to as ultrasonic resonators, separators or filters or acoustic wave separation (AWS).
perfusion system becomes a powerful solution for perfusion cultures in SU continuous or semicontinuous operations. The Cytoperf has an adjustable acoustic frequency to minimize heat energy to be dissipated and requires no complex cooling loop (which can create a gradient temperature across the field). This gamma sterilized or autoclavable single use devise employs a unique nonrecalculating loop in its movement of cells. Because of its size, a battery of such units would be required for most biomanufacturing applications.

**Spinfilters**

Internal and external spinfilters of various sorts have been successfully used for decades. They are primarily intended to induce a tangential flow of medium across a cell filtering screen to prevent it’s clogging during perfusion culture. The filtering mechanism has historically been made from layers of cleanable stainless steel mesh. Sartorius BBI Systems (BBI) provides a range of external spin filters for use with any type of cell culture and has recently developed a new, single-use system (Spinfilter P) that can be used with stirrer shafts of varying sizes. The main body of this disposable unit is polycarbonate and features a filtering open mesh component of highly specialized polyester polyethylene terephthalate ployester (PETP) monofilament fabric (Table 2C) [78].

**Conclusion**

Continuous processing is a well-established and fundamental mode of modern manufacturing. The real key to the successful implementation of CP approaches in biomanufacturing is the recent progress made in process monitoring, a number of advanced process control developments and such OpEx goals as increased process knowledge and critical process parameter establishment. In fact, such developments, as well as improvements in culture media and mass transfer techniques, were required to enable even consideration of application of CP to biomanufacturing generally. Many are now understanding the power of such perfusion-culture benefits as reduced reactor residency and improved product consistency as well as such CP benefits as simplified production trains and reduced handling of intermediates. These features complement the operational efficiencies of SU systems as well as contribute to a greatly reduced cumulative processing time and personnel activity in production. Recent trends in biomanufacturing demand synergize with both single-use and CP values and features. We are witnessing a growing interest in continuous biomanufacturing, and implementation is being enabled by the fact that number of biopharmaceutical sponsors are already implementing significant CP-compatible SU technologies and operations.

**Future perspective**

The modularity and integral gamma irradiation sterility of SU systems, combined with the reduced footprint and simplicity of CB, yield significant promise for the future. Especially when considered in light of heightened process understandings, PAT and QbD support of new monitoring approaches and design-space establishment. Such developments are even allowing consideration of such paradigm shifts as real-time, continuous quality and process verification supporting designs. Industry innovators are now establishing intensified processes in vertically integrated, closed and disposable continuous upstream bioproduction systems [79]. As SU perfusion bioreactors, or perfusion-enabling add-ons, become mainstream – we will see more continuous operations being implemented. The current goal for many is to implement SU continuous processes in ‘Factories-of-the-Future’ consisting of pre-engineered, modular and turn-key multiproduct manufacturing facilities. Assembled on the sponsor’s site, they would provide qualified, cGMP-compliant preassembled skids consisting of environmentally sustainable approaches to flexible manufacturing. For many platforms, such designs can also be imagined in either ready-to-use, microenvironment-based flexible factories or preassembled streamlined suite pods promoting closed processing within open-production halls or unclassified ‘ballroom’ controlled nonclassified suites. Such facilities might possess advanced inline testing technologies, eventually establishing a global, enterprise process control integrating the scheduling and management of such activities as:

- Raw material supply;
- Media and buffer preparation;
- Equipment maintenance and calibration;
- Facility, mechanical systems and process control systems.

Some envision that this all just might someday support the rapid, local establishment of flexible, continuous biomanufacturing operations with real-time release of even unit doses.

**Financial & competing interests disclosure**

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.
Executive summary

**Single-use systems**
- Many valuable features are provided by single-use (SU) including lower initial investment, facility and operating costs.
- Many upstream operations are supported by SU including media and process liquid preparation and cell culture for production.

**Continuous processing**
- Valuable features provided by continuous processing include increased product quality and reductions in construction and operating costs.

**Continuous biomanufacturing**
- Valuable features provided by continuous biomanufacturing (CB) include reductions in classified suite extent, personnel requirements and operating steps.

**Benefits of SU continuous biomanufacturing**
- The benefits of SU CB include heightening processing parameter consistency and increasing process efficiency/capability.

**Perfusion culture**
- Valuable features provided in perfusion include significantly reduced product-reactor residency duration and a dramatic increasing in reactor-volumetric productivity.

**SU perfusion bioreactors**
- SU equipment and technologies provide perfusion complementing/enabling features in continuous biomanufacturing implementations.
- Commercially available SU and hybrid production-scale perfusion-support has appeared for most any process format, platform or mode.
- Environmental objectives are supported as CB reduces the equipment to be clean-in-place/steam-in-place (CIP/SIP) and their required services and energy consumption.
- Commercialized SU perfusion equipment includes mechanically agitated suspension, hollow-fiber, floating-filter and packed-bed reactors.
- SU accessories supporting perfusion culture include hollow fiber exchange, continuous-flow centrifugation and acoustic wave separation.

**References**

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Recent improvements in expression vectors, process development and facility construction support demands for cost–effective and flexible biomanufacturing facilities. This article explains how the need for simple but flexible facilities is fed by demand for globalization of the biopharmaceutical industry, patent expirations and a shift in new product types.


The costs involved a continuous manufacturing process to synthesize API and formulate it into tablets are estimated with, for example, raw material cost, production yield and API loading varied over broad ranges. This article models how even when yields in the continuous case are lower than in the batch case, savings can still be achieved because other savings compensate.


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This study reports on successful integration of perfusion culture and periodic counter-current chromatography (PCC). Two examples are presented: a MAb and an rEnzyme. High-density perfusion CHO cell cultures were maintained for more than 60 days achieving productivities much higher than current processes.

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• This paper introduces the reasons why as perfusion/modification of reactors are contiguously combined with other enabling technologies such as single-use mixers and PCC, the design of closed, disposable and continuous biomanufacturing systems is firmly being realized.


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