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Jungbauer, Alois; Satzer, Peter; Duerauer, Astrid; Azevedo, Ana; Aires-Barros, Raquel; Nilsson, Bernt; Farid, Suzy; Goldrick, Stephen; Ottens, Marcel; More Authors

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Alois Jungbauer [a,b,*], Peter Satzer [a,b], Astrid Duerauer [a,b], Ana Azevedo [c], Raquel Aires-Barros [c], Bernt Nilsson [d], Suzy Farid [e], Stephen Goldrick [e], Marcel Ottens [f], Mattia Sponchioni [g], Hector Marcelo Fernandez Lahore [h]

[a] Austrian Centre of Industrial Biotechnology, Vienna, Austria
[b] Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria
[c] Institute of Bioengineering and Biosciences, Associate Laboratory i4HB- Institute for Health and Bioeconomy, Department of Bioengineering, Instituto Superior Tecnico, Universidade de Lisboa, Portugal
[d] Department of Chemical Engineering, Lund University, Sweden
[e] Department of Biochemical Engineering, University College London, United Kingdom
[f] Department of Biotechnology, Technical University Delft, the Netherlands
[g] Department of Chemistry, Materials and Chemical Engineering, Politecnico di Milano, Italy
[h] Unit Environmental and Industrial Biotechnologies, Luxemburg, Institute of Science and Technology, Luxembourg

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ABSTRACT

The main objectives of bioprocesses are to reliably deliver drugs in a relatively short time frame with high quality within a tight regulatory framework. Bioprocesses are highly complex, the level of automation is moderate, and there is constant pressure to improve efficiency and costs. In addition, climate change and resource scarcity mandate a reduction in the environmental footprint of bioprocesses and production facilities. In the biopharmaceutical industry, two extreme production scenarios are applied: a fully disposable factory with the characteristics of full flexibility and speed, or a fixed large-scale plant with high capacity. Forward-looking solutions and ideas will be discussed how to combine new processes and environmental friendliness for the benefit of the patient, security of supply and profitability. The concept will be extended to large scale production of proteins for food and non-pharma applications, *e.g.*, in material science and a roadmap towards a future plant will be laid out.

1. The global context

Pharmaceutical, including biopharmaceutical, is the second strongest industrial sector in Europe in terms of exports[1]. The production of biopharmaceuticals is a key industry in Europe with high added value. Currently, more than half of the pharmaceutical market in Europe and the US is biopharmaceuticals. 60% of these are antibodies or antibody-derived molecules, which are very complex molecules consisting of a large number of molecular entities that are affected by process conditions. However, the biopharmaceutical sector is sensitive to production expense, as process and manufacturing costs are much higher than for traditional pharmaceuticals.

The biopharmaceutical sector in Europe is being strongly challenged by other countries such as the U.S., India, and especially the Chinese government. There, the pharmaceutical sector, including the biopharmaceutical industry, is explicitly on the agenda of the recently launched "2025 Made in China" initiative. China's 13th Five-Year Plan[2] calls for the biotechnology sector, which includes the biopharmaceutical sector, to account for more than 4% of gross domestic product by 2020, and for there to be 10 to 20 life science parks for biomedicine and biopharmaceuticals with production exceeding $1.5 billion. China has more than 100 life science parks scattered across the country, operated by local governments and luring companies with tax breaks and subsidies. It is estimated that the state has already invested more than $100 billion in the life sciences sector[3].

The extremely long development time of new products is unique in the biopharmaceutical industry sector. It can exceed 10 years, but the patent life is only 18 years. In addition, only 10% of newly discovered products survive clinical trials. Therefore, a fast fail/speed to market strategy has become prevalent in the pharmaceutical and biopharmaceutical industries [4]. Maximum revenue is only achieved when new biopharmaceutical products are brought to market as quickly as possible. In reality, this pressure leads to the launch of new biopharmaceutical products with non-optimized manufacturing process

* Corresponding author at: Austrian Centre of Industrial Biotechnology, Muthgasse 18 1190 Vienna, Austria.
E-mail addresses: alois.jungbauer@boku.ac.at (A. Jungbauer), astid.duerauer@boku.ac.at (A. Duerauer).

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conditions using traditional batch processes that are relatively fast to develop compared to continuous. The education and training of scientists and engineers in the field of biopharmaceutical manufacturing helps industry to close the gap between launch of products with non-optimized process and the need to enter the market. Downstream processing is a central part of biomanufacturing and young scientists in this sector are urgently needed and are a major contribution to keeping the European biopharmaceutical industry competitive.

2. State of the art of the technology

The future of global bioprocessing demands are flexible, scalable solutions for a rapidly changing landscape of the biopharmaceutical industry[5]. In addition, minimizing the impact on the environment in the face of climate change is the need of the hour and time. Two extreme production scenarios exist: on the one hand, fully disposable factories offer flexibility and enable high speed and rapid change of products. On the other hand, large stainless-steel plants have been designed for high capacity and economies of scale. Such plants are mainly dedicated to one product but solutions for a medium-scale production are also common. The small disposable factory presents the solution for production of preclinical or clinical material and for contract manufacturing where the product is often switched. Integrated continuous biomanufacturing has been already considered for fast production of smaller amounts of material but its real advantage is in the full-scale manufacturing offering a substantial reduction of costs and environmental impact potentially using the small flexible facility paradigm[6,7]. In addition, a continuous manufacturing unit can be controlled and automated[8].

In view of the metric Process Mass Intensity (PMI, amount of material required for a defined amount of product, *e.g.*, kg/kg)[9] adopted by the ACS green chemistry group conventional manufacturing of biologics is not efficient. PMI in antibody manufacturing is in the range of 7000 kg/kg product, whereas for small molecules the PMI is in the range of ≈ 300 kg/kg product. In the case of biologics, the PMI is driven by water consumption, with downstream processing being the main driver for high water and material consumption. Electricity used to power the cleanroom infrastructure are the major contributor to the environmental footprint. American Chemical Society Green Chemistry Institute Pharmaceutical Roundtable has estimated 22.7 tons CO_2 eq per 1 kg bulk drug substance, *e.g.* antibody using single use technology at a 2000 L bioreactors scale[13] Water and energy reduction is the area where continuous downstream processing can make a major contribution due to better resources efficiency and reduction of floor space[10–12].

A manufacturing process in the bio-industry consists of upstream processing (cultivation of cells in the bioreactor) and downstream processing (recovery of the product). After primary recovery by centrifugation and/or microfiltration, the product is usually further purified in a series of chromatography steps interconnected with ultrafiltration/diafiltration and other conditioning steps. In traditional processing, all of these steps are mostly performed in a batch-wise manner, most often in a fixed time mode, with little or no digitization or automation.. For licensed products using this production process, control is minimal and, in most cases, samples are taken at each step and analyzed offline[14]. Real-time in-process control is limited to checking pressure, flow rate, or buffer composition, but product quality or quantity/titer is not analyzed. Over the past decade, the regulatory framework has been very clear that process characterization is ultimately driven by the principle of "Quality by Design" (QbD). This requires improved process understanding, scale-down tools, Process Analytical Technology (PAT) tools, multivariate data analysis, machine learning algorithms and control strategies to enable a shift from fixed processes to adjustable processes within a design space based on desired product quality and performance [15–19].

The current trend in the industry is to gradually move from batch processes (Fig. 1A) to integrated, continuous downstream processing strategies to minimize the risks of process failures (Fig. 1D). Thus, the next step for the bioindustry is to adopt straight-through or seamless downstream processing. In this mode of operation, the product from one unit can be processed directly by the following unit without the need for conditioning or intermediate storage. The process is simplified, but still occurs in batch (Fig. 1B). Another attempt to gradually introduce continuous downstream processing is to test periodic counter current loading[20] an old technology applied for water desalination[21], which simulates a quasi-continuous process in chromatography[22–24]. This process could be a crucial step for future continuous downstream processing (Fig. 1C).

Current progress is hampered by a shortage of trained engineers and scientists who can design and control continuous biomanufacturing processes. We have formed an Innovative Training Network (ITN) named "Continuous downstream processing of biologics" (CODOBIO, https://www.codobio.eu/) with focus on addressing critical gaps in research training to enable advances in continuous downstream processing. An expert consortium of ten industry partners, nine universities, a research institution, a regulatory agency, and a consulting firm has developed a research and training program that addresses the most pressing issues in continuous downstream processing. These are: (1) process control and modelling (including economic modelling), (2) miniaturization, scale-up and scale-down of unit operations and (3) process design and development of integrated continuous downstream processes.

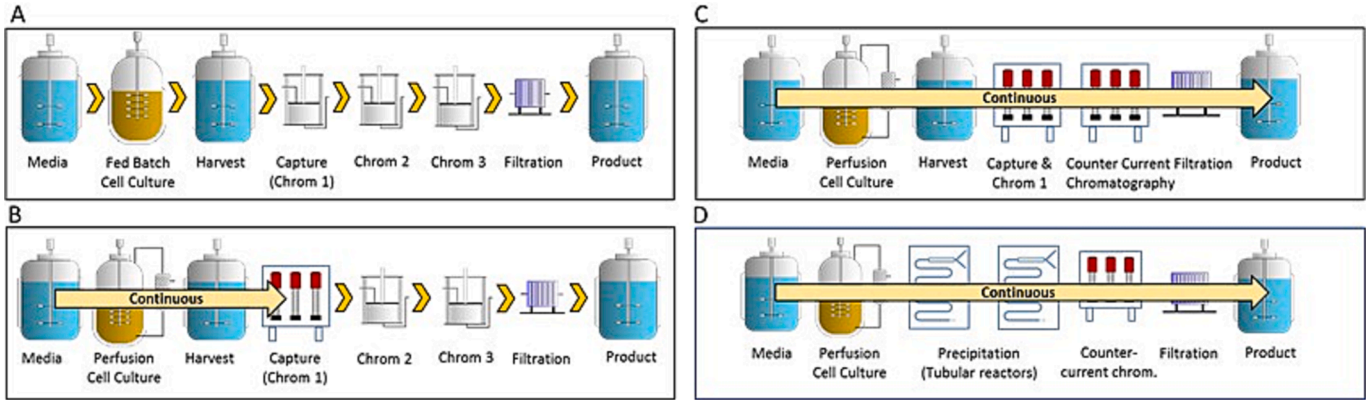

Fig. 1. Overview of transition from batch downstream processing to a full end to end continuous manufacturing concept; A: Full batch production, B: Continuous Upstream Processing with pseudo-continuous capture, rest: batch downstream processing, C: Continuous Upstream Processing and fully integrated downstream processing with pseudo-continuous chromatography D: Fully integrated continuous manufacturing with continuous up- and downstream processing using fully continuous tubular reactors.

Recommendations are made on how the results of this research consortium can be implemented in the development of new products and processes using the concept of integrated biomanufacturing and where we see an urgent need for further research.

3. Challenges and opportunities

In recent years, the transition to continuous manufacturing has been recognized as the next step in further optimizing production processes in the bioindustry, particularly in the manufacture of biopharmaceuticals, nutraceuticals, cosmeceuticals, and enzymes. Continuous manufacturing in the bioindustry offers new opportunities to increase productivity, reduce environmental impact, improve product quality and consistency, and dramatically reduce manufacturing costs, making biologics accessible to a much wider population, including those in non-privileged countries. Continuous manufacturing will lead to further automation in the biologics industry[14]. Process development and technology transfer to production will become more complex, individual steps will be fully integrated, and rigorous in-process control will be required. The significant reduction in the space required for the process will allow the manufacturing process to be carried out in a very small space, *e.g.*, in a container, which can be transported to any location in the world supporting global market mandates such as "2025 Made in China[25]. This drastically simplifies process transfer between different manufacturing sites. The characteristics of integrated continuous manufacturing processes, the requirements needed to implement them, and the results achieved through their implementation are shown in Fig. 2.

We see the following challenges in converting batch manufacturing into continuous.

1. In industry there is a "Burden of Change" which often delays decisions. A lot of companies do not want to be in the role of a pioneer and often the business model is not recognized.
2. Scale-down adaptation of existing continuous technologies is difficult, and models and equipment are not readily available.
3. Skilled engineers and scientists capable of installing, operating, and troubleshooting the equipment needed for continuous downstream processes are not yet available in the industry, which could slow the move toward continuous manufacturing. The same is true for all issues related to the digitization of processes, the use of advanced online monitoring tools, including the possibility of implementing Big Data and machine learning tools.

CODOBIO's research and training program has developed methods and models for the implementation of innovative continuous manufacturing processes in the bioindustry. However, this review covers recommendations and solutions, which go beyond the research and training program.

4. Scale-down

Scale-down of continuous bioprocesses is a topic that researchers and industry feel uneasy about because it breaks new ground. A scaled-down version of an integrated bioprocess is needed for process development, process characterization as part of a QbD strategy, virus clearance studies, and often to produce a specific amount of material to compare whether materials produced in batch and continuous mode are the same. Although there is ample of knowledge to translate batch operations into continuous but there is not enough industrial experience available when operating parameters can be estimated from a conventional batch experiment or when continuous operation is required. This is much clearer for upstream processing than for downstream processing. A batch bioreactor cannot be used to emulate continuous production, although semi—batch methods have been proposed to mimic a perfusion bioreactor[26]. Often, operating parameters and parameters for modeling unit operations in downstream processing can be estimated from small-scale laboratory experiments. For micro-ultrafiltration, chromatography, and precipitation, this has already been practiced and models are available. The design of simulated moving bed can be exclusively made from parameters estimated by batch experiments. Likewise, we are capable of designing a counter current loading of a continuous multi-step filtration from batch data.

A typical manufacturing campaign in continuous integrated biomanufacturing is designed to last 30 days, and for some companies as long as 60 days[27]. While we are able to design a process using operating parameters estimated from batch experiments, the impact of surface aging, filter clogging and fouling can only be measured when running a process for a certain period of time. It is not possible to evaluate these effects at full or pilot scale, because it is too costly. Therefore, a scaled-down version that is representative of the full-scale must be used. In a scaled-down version, many more process variations can be tested, and the material consumption is reduced [28,29].]. However, steps that have an aging effect would still be required to operate for long durations to match performance in some unit operations (*e.g.* SPTFF with matching flux and membrane loadings). The advantage of an integrated continuous biomanufacturing (ICB) process is the

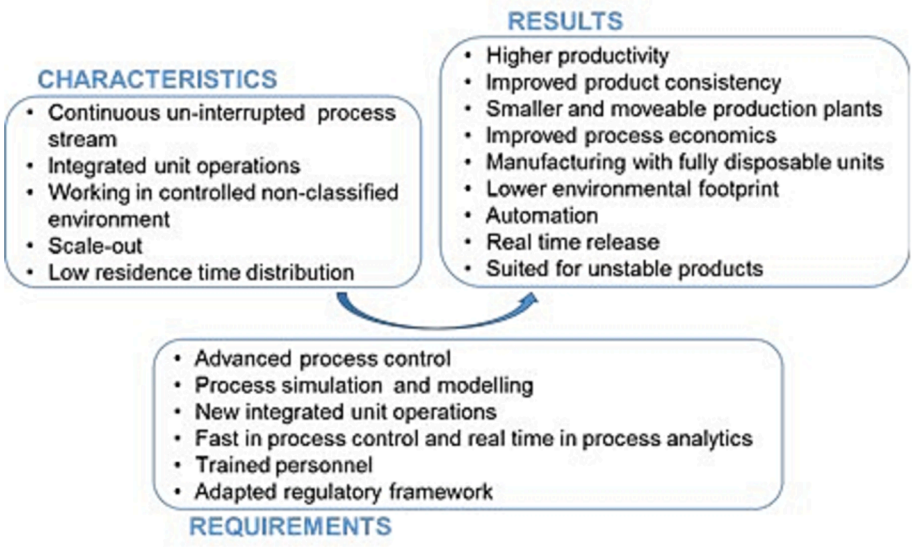

Fig. 2. Characteristics of integrated continuous manufacturing processes, requirements needed to implement them, and results achieved by their implementation.

reduction of scale by approximately a factor of 10. Therefore, a typical laboratory plant is already a pilot plant when operated in continuous mode, and a pilot plant in batch mode, once converted to a continuous plant, can serve as a production scale plant. So, when further downsizing is required, we must deal with very low flow rates, narrow tubes, and often with self-built equipment.

Currently, we see challenges in the scale-down of continuous integrated biomanufacturing:

1. Scaled-down equipment is not readily available with automation solutions for connecting process equipment.
2. Integrated process skids are not available.
3. Sensors and actuators are not available on a small scale.
4. Certain unit operations cannot be scaled down without changing geometry and design, *e.g.*, disk stack centrifuge.
5. In the ultimate scale-down of microfluidics, the hydrodynamics are radically changed, and if mixing or turbulent flow is required, the extremely scaled-down devices are not representative any more for laboratory and full scale.

In the frame of the CODOBIO project devices have been developed and their role of micro- and millifluidics in process development was evaluated. It has been determined to which extent mixing behavior in microtiter plates can be extrapolated to small and large-scale reactors [30]. In the case of chromatography, it is possible to use microfluidic devices to estimate equilibrium parameters (Fig. 3)[31]. It is not yet clear to what extent kinetic parameters and column dynamics can be mimicked[32].

In addition, it is possible to use such microfluidic devices as at-line detectors for critical impurities[33–35] or on-line sensors in micro-chromatography columns[36]. Microfluidics also offers the possibility to integrate upstream and downstream processes and to combine bioreactor, cell lysis if needed and capture in one device[37].

When turbulent flow is required, microfluidics is no longer practical. Millifluidics devices can then be used[38,39]. They offer the advantage of lower material consumption compared to a conventional laboratory system and the ability to work under turbulent flow conditions and do not require expensive cleanroom microfabrication techniques[40]. A typical example is protein precipitation, where fast mixing is required and mixing influences, yield, filterability, and purity of the product [38,39].

When scale-up with constant volumetric power input is considered, direct scale-up of mixing studies from microtiter plates to stirred tank reactors in downstream processing is possible. A power input of 300 W/m^3 is achievable[30]. This does not apply to upstream processing, where much higher power consumption is often required, and one of the scale-up parameters is the lumped mass transfer parameter k_La[41].

Recommendations: The most complex scale-down of continuous downstream processing is liquid–solid separation and virus clearance. It is very time and material consuming to show how the performance changes over time and when a process must be terminated. This is uncharted territory and has not yet been satisfactorily resolved. Therefore, more research must focus on this aspect. We suggest that the milli- and microfluidic devices developed in CODOBIO are a good starting point for this purpose.

For mixing operations in downstream processing and for keeping a reactor just in suspension we can recommend process development in microtiter plates because it is possible to directly scale-up to stirred tank reactors. Together with micro and millifluidics this is recommended as preferred method for high throughput parameter estimation as already suggested previously.

5. New materials and technologies

The core unit operations of downstream processing for production of biopharmaceuticals are a combination of chromatography, ultra- and microfiltration, virus inactivation by lowering pH or addition of viricidal agent and virus filtration. Rarely are other unit operations employed. Chromatography has been successfully converted into a continuous operation either in the form of counter current chromatography (with two, three or more columns), twin column chromatography or multi-column solvent gradient chromatography. Simplest conversion of a batch filtration to continuous mode is a tandem filtration, although more sophisticated versions are available such as two-stage filtration, countercurrent filtration or single-pass filtration[42], but latter one suffers from limits in scale-down and therefore it is difficult to implement in a small-scale unit[43,44].

Countercurrent chromatography was also developed to improve column utilization and achieve high productivity to overcome the sweet spot in conventional batch chromatography where there is an optimum between column utilization and productivity[45–51]. By overloading one column and directing the effluent to a second, it is possible to run chromatography columns with high productivity and column utilization. The high column utilization is required to reduce buffer consumption and chromatography material for a given amount of product to be processed. Countercurrent loading meets the requirements, but at the expense of the complexity of the operation. When a flow gradient is used during batch chromatography loading, it is possible to further increase column utilization along with productivity, thereby significantly reducing buffer consumption and chromatography material compared to countercurrent loading. Equipment complexity is much lower compared to countercurrent loading. Installing a controller along with soft sensors for the product can even compensate for column aging associated with decreasing capacity[52,53].

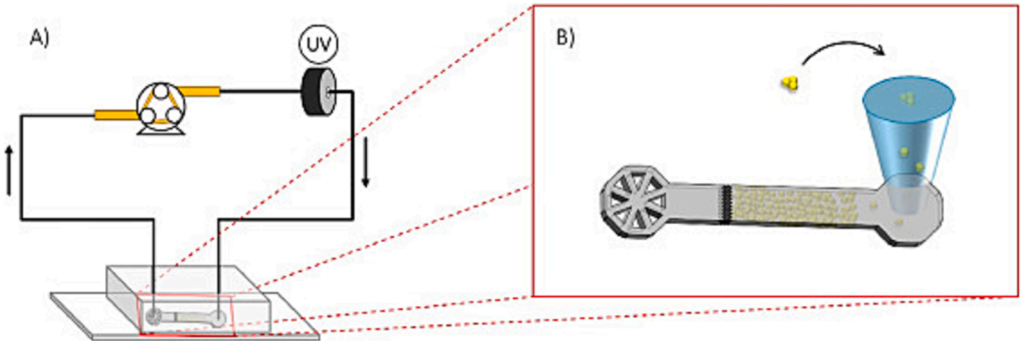

Fig. 3. Microfluidic experimental setup for the determination of protein adsorption isotherms. A) Schematics of the setup. Yellow lines represent the PharMed® BPT tube that is connected to the peristaltic pump; black lines represent the PEEK tubing. Arrows show the direction of fluid flow through the system. B) Zoomed in image of the microfluidic chip with schematics for resin loading into the microchip. The resin is loaded using a micropipette tip and the chromatographic beads are trapped by the frits further down the channel, published with permission from Silva et al. [31]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The continuous counter current chromatography is not a real continuous operation, the feed flow is continuous, but the effluent/eluate flow is periodically. Fully continuous chromatographic operations such as annular chromatography[52,53] or true moving bed chromatography have been proposed and extensively tested but never entered industrial practice. A true operation is the continuous countercurrent tangential chromatography system from ChromaTan in which the adsorbents in the form of a resin slurry flows sequentially through a series of static mixers and hollow fiber membrane modules to perform the steps of binding, washing, elution, stripping and equilibration in continuous operation[45,54,55]. The system is currently available as bench scale units.

A hypothetical concept has been around for many years, the moving belt adsorber[56]. The adsorbent is a moving belt with adsorptive properties for the product dipping in baths containing the feed, washing or elution solutions. Hereby a fully continuous operation could be established. A challenge is the absorber material, it must fulfill certain mechanical requirements such as tensile strength but also surface properties such a sufficient porosity and chemical functionality to accommodate fast and high product binding and releases. Also, when a riser using adsorbent porous beads, a device which comes very close to a true moving bed, is applied for continuous chromatography, the mechanical and chemical properties of the beads are crucial for the performance. These new methods together with continuous countercurrent tangential chromatography system are expected to accommodate the recovery and partial purification of unclarified feedstock *e.g.*, fermentation broth or cell culture in real continuous mode.

Fibrous adsorbents with high sorption capabilities are a solution for mechanically-robust moving belts. Although with a woven structure created out of nylon/dextran composite microfibers, a promising material[57] has been found and it has been realized that convective flow must be employed to fully exploit the potential of such a continuous operating adsorber belt[58].

Another possibility to generate a fully continuous chromatography system is the combination or extension of a true moving bed with a countercurrent conductor and a riser which includes also steps required for washing, elution and regeneration of the adsorbent beads analogous to the packed bed operation (Fig. 4).

Devices such as the moving belt or the fluidized bed riser are still self-made and therefore the implementation in industry will not advance. CODOBIO contributes new technologies for fully continuous downstream processing avoiding a periodic eluent stream[59].

An essential component of bioproduction, both on a laboratory and production scale, is buffer management[60]. Buffers must be prepared and stored, their volume monitored, and replenished as needed, which can be very labor intensive and a large part of the process. With this in mind, a buffer management system was developed for lab-scale processing that includes automated ordering, preparation, and delivery of buffers[61]. The buffer system was demonstrated with the integrated continuous downstream process and all required buffers were automatically delivered by the buffer management system during a 10-day run[62,63].

Recommendations: It has been proven that concepts for fully continuous chromatography work, but it is necessary to bring such system to a GMP level to be acceptable by industry. It is now well understood how mechanical strength and adsorptive properties in continuous conveyor belt chromatography can be combined with a convective flow and therefore a truly continuous chromatography system is on the horizon. Tailoring process operational parameters and adsorbent design is further necessary to run a fluidized bed riser adsorption system. Whenever such a fully continuous adsorption and desorption is required then the conveyer belt and fluidized bed riser adsorption system should be taken into consideration. Efficient buffer management has been tested in laboratory and pilot scale. Efforts to demonstrate such technologies at higher TRL levels are guaranteed.

When equipment simplicity is of interest then flow gradients during loading are recommended. In such a way the conventional batch chromatography can be used, but similar productivity and column utilization as in counter current chromatography can be achieved[52,53,64]. By running two columns in a staggered fashion a quasi-continuous operation can be realized.

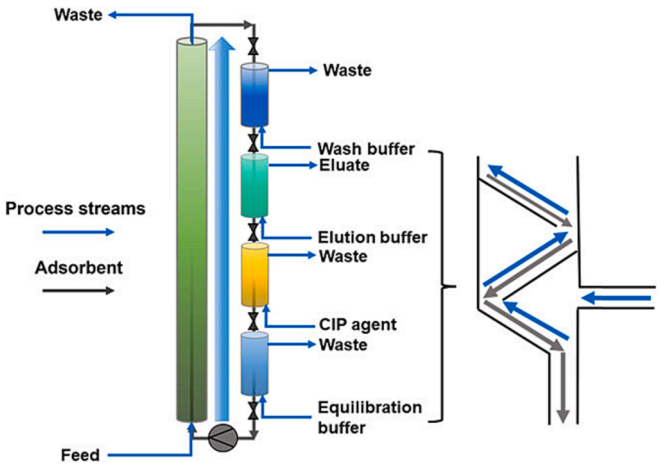

Fig. 4. Process scheme for fluidized bed riser adsorption system including counter-current contactor design for elution, CIP, and re-equilibration stages. CIP, cleaning-in-place, reproduced with kind permission from Herlevi et al. [59].

6. Process characterization

The legal framework for continuous integrated biomanufacturing is much clearer now. The ICH guidelines Q13 on continuous manufacturing of drug substances and drug products has been adopted by the European Medicines Agency and by the US Food and Drug Administration in 2023 after several years of consulting. Together with the Process Analytical Technology (PAT) initiative and real time release guidelines, the way is paved for a completely new way of manufacturing biopharmaceuticals. Continuous operation allows the consequent implementation of monitoring and control and process automation[65]. The incentive of the health agencies together with industry was to change the way of production with the benefit of higher consistency, more robustness, and reduced costs. This has been claimed for many years and already shown last decade but now the phase of implementation in industry is commencing. With the reduced cost in continuous biomanufacturing also reduced environmental footprint is achieved, which in light of the EU Green Deal goals for 2030 and 2050 and the connected necessity of substantially reducing our carbon footprint/environmental footprint is a clear motivation for change.

Several important scientific topics covered by the ICH Q13 guideline have been covered by CODOBIO and anticipated before even the first draft was released. ICH Q13 addressed batch definition, control strategies with state control, process dynamics, material characterization and control, equipment design and system integration, process monitoring and control, material traceability and diversion, and process models. In the regulatory section also lifecycle management is included.

Modelling the residence time distribution of an entire process chain helps to understand the process dynamics and is a decision-making tool for start up and shutdown of an integrated manufacturing process based on a mathematical algorithm[66,67]. It can help to design better processes with faster start up and shutdown stages as well as to aid for the definition of a production batch. The residence time distribution helps to demonstrate equivalency between different scales and to interpret small scale operations, where a certain unit operation is not available at small scale. It is also a decision-making aid for time required to resume product collection after a process has run out of specification. In

CODBIO, we also experienced that an inert tracer is very difficult to find. Especially when porous media such as packed beds with porous chromatography media or porous membranes are used, RTD must be assessed with a tracer with similar mass transfer and adsorption properties as the product[68].

The fundamental understanding of modeling tools for the monitoring and control of integrated chromatographic purification processes and of the interactions among different steps in terms of product quality and productivity was also investigated and demonstrated with a fully end to end integrated process for antibody production[69–73].

The modeling tools will contribute to a significant effort being made in the biotech industry to develop digital twins that enable faster development, optimization and performance of the process[74,75].

The consequent analysis of process economics also benefits the understanding of the environmental footprint of a process. It is obvious that a reduced material consumption, a higher productivity and a small floor space required for the production process will automatically lead to a better environmental footprint.[76–78].

Recommendations: Modelling is the link to scale-down and how representative the scale-down is indeed. Without a modeling concept it is almost impossible to develop an end-to-end integrated manufacturing process and to benefit from its economics. It is recommended that a residence time distribution of the process is established at a very early stage to enhance the process understanding. Once the models for RTD are in place, they can be used also for other products, because the model is universal and only a handful of unit operations are used in biomanufacturing. A full life cycle analysis may be too costly and impede the process development time. A shortcut method is an assessment of the environmental footprint by simple parameters such as process mass intensity or energy consumption based on output. The carbon footprint is a more difficult parameter to assess and in biotechnology often related to water consumption.

7. Conclusion

Continuous downstream processing consists of the combination of different unit operations which are already present in batch wise production. Converting these unit operations into a continuous mode often results in a pseudo-continuous operation with cyclic behavior. Alternative concepts such as continuous conveyor belt adsorption or fluidized bed riser adsorption are in place and can be considered in the future to establish fully continuous processes without cyclic behavior. The consequent application of milli- and microfluidics is one way to overcome the large material consumption required for process development. Microfluidics also provides the ability to detect critical impurities. Process and economic modeling help to understand the process. The consequent integration of all unit operation is only possible by applying process monitoring and control, but as a reward, the process is then automated.

CRediT authorship contribution statement

Alois Jungbauer: Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Peter Satzer:** Supervision, Project administration, Investigation. **Astrid Duerauer:** Supervision, Methodology, Formal analysis. **Ana Azevedo:** Writing – review & editing, Supervision, Methodology, Investigation. **Raquel Aires-Barros:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Bernt Nilsson:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Suzy Farid:** Writing – review & editing, Supervision, Methodology. **Stephen Goldrick:** Supervision, Project administration, Methodology, Formal analysis. **Marcel Ottens:** Writing – review & editing, Supervision, Software, Resources, Methodology. **Mattia Sponchioni:** Writing – review & editing, Validation, Supervision, Software, Methodology. **Hector Marcelo Fernandez Lahore:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

[1] E.c.p. release, European commission press release: Factors influencing industrial competitiveness in the EU,, http://europa.eu/rapid/press-release_MEMO-14-527_en.htm?locale=EN, (2014).
[2] M.i.C.p.u.t.b. manufacturing, https://web.archive.org/web/20180725113438/https://gbtimes.com/made-china-2025-plan-unveiled-boost-manufacturing.
[3] S. Ellis, Biotech booms in China, Nature 553 (2018) S19–S22.
[4] R. Khanna, I. Guler, A. Nerkar, Fail often, fail big, and fail fast? Learning from small failures and R&D performance in the pharmaceutical industry, Acad. Manage. J. 59 (2016) 436–459.
[5] K.B. Konstantinov, C.L. Cooney, White paper on continuous bioprocessing May 20–21, continuous manufacturing symposium, J. Pharm. Sci. 104 (2015) (2014) 813–820.
[6] J. Walther, R. Godawat, C. Hwang, Y. Abe, A. Sinclair, K. Konstantinov, The business impact of an integrated continuous biomanufacturing platform for recombinant protein production, J. Biotechnol. 213 (2015) 3–12.
[7] O. Khanal, A.M. Lenhoff, Developments and opportunities in continuous biopharmaceutical manufacturing, MAbs 13 (2021).
[8] A. Dürauer, A. Jungbauer, T. Scharl, Sensors and chemometrics in downstream processing, Biotechnol. Bioeng. (2023).
[9] S.R. Madabhushi, J. Gavin, S. Xu, C. Cutler, R. Chmielowski, W. Rayfield, N. Tugcu, H. Chen, Quantitative assessment of environmental impact of biologics manufacturing using process mass intensity analysis, Biotechnol. Prog. 34 (2018) 1566–1573.
[10] K. Budzinski, M. Blewis, P. Dahlin, D. D'Aquila, J. Esparza, J. Gavin, S.V. Ho, C. Hutchens, D. Kahn, S.G. Koenig, R. Kottmeier, J. Millard, M. Snyder, B. Stanard, L. Sun, Introduction of a process mass intensity metric for biologics, N. Biotechnol. 49 (2019) 37–42.
[11] S.R. Madabhushi, N.D.S. Pinto, H. Lin, Comparison of process mass intensity (PMI) of continuous and batch manufacturing processes for biologics, N. Biotechnol. 72 (2022) 122–127.
[12] A.L. Cataldo, D. Burgstaller, G. Hribar, A. Jungbauer, P. Satzer, Economics and ecology: Modelling of continuous primary recovery and capture scenarios for recombinant antibody production, J. Biotechnol. 308 (2020) 87–95.
[13] K. Budzinski, D. Constable, D. D'Aquila, P. Smith, S.R. Madabhushi, A. Whiting, T. Costelloe, M. Collins, Streamlined life cycle assessment of single use technologies in biopharmaceutical manufacture, N. Biotechnol. 68 (2022) 28–36.
[14] V. Chopda, A. Gyorgypal, O. Yang, R. Singh, R. Ramachandran, H. Zhang, G. Tsilomelekis, S.P.S. Chundawat, M.G. Ierapetritou, Recent advances in integrated process analytical techniques, modeling, and control strategies to enable continuous biomanufacturing of monoclonal antibodies, J. Chem. Technol. Biotechnol. 97 (2022) 2317–2335.
[15] M.N. São Pedro, M.E. Klijn, M.H.M. Eppink, M. Ottens, Process analytical technique (PAT) miniaturization for monoclonal antibody aggregate detection in continuous downstream processing, J. Chem. Technol. Biotechnol. 97 (2022) 2347–2364.
[16] L. Rolinger, M. Rüdt, J. Hubbuch, A critical review of recent trends, and a future perspective of optical spectroscopy as PAT in biopharmaceutical downstream processing, Anal. Bioanal. Chem. 412 (2020) 2047–2064.
[17] M. Rüdt, T. Briskot, J. Hubbuch, Advances in downstream processing of biologics – Spectroscopy: An emerging process analytical technology, J. Chromatogr. A 1490 (2017) 2–9.
[18] S. Zobel-Roos, A. Schmidt, F. Mestmäcker, M. Mouellef, M. Huter, L. Uhlenbrock, M. Kornecki, L. Lohmann, R. Ditz, J. Strube, Accelerating biologics manufacturing by modeling or: Is Approval under the QbD and PAT approaches demanded by authorities acceptable without a digital-twin? Processes 7 (2019).
[19] A. Schmidt, H. Helgers, L.J. Lohmann, F. Vetter, A. Juckers, M. Mouellef, S. Zobel-Roos, J. Strube, Process analytical technology as key-enabler for digital twins in

continuous biomanufacturing, J. Chem. Technol. Biotechnol. 97 (2022) 2336–2346.
[20] B.H. Arve, A.I. Liapis, Biospecific adsorption in fixed and periodic countercurrent beds, Biotechnol. Bioeng. 32 (1988) 616–627.
[21] G. Carta, R.L. Pigford, Periodic countercurrent operation of sorption process applied to water desalination with thermally regenerable ion-exchange resin, Reactive Polym. Ion Exchangers, Sorbents 6 (1987) 51–52.
[22] B. Somasundaram, K. Pleitt, E. Shave, K. Baker, L.H.L. Lua, Progression of continuous downstream processing of monoclonal antibodies: Current trends and challenges, Biotechnol. Bioeng. 115 (2018) 2893–2907.
[23] F. Steinebach, T. Müller-Späth, M. Morbidelli, Continuous counter-current chromatography for capture and polishing steps in biopharmaceutical production, Biotechnol. J. 11 (2016) 1126–1141.
[24] A.L. Zydney, Continuous downstream processing for high value biological products: A Review, Biotechnol. Bioeng. 113 (2016) 465–475.
[25] J. Xu, X. Xu, C. Huang, J. Angelo, C.L. Oliveira, M. Xu, X. Xu, D. Temel, J. Ding, S. Ghose, M.C. Borys, Z.J. Li, Biomanufacturing evolution from conventional to intensified processes for productivity improvement: a case study, MAbs 12 (2020).
[26] M. Pappenreiter, B. Bayer, M. Logarušić, B. Sissolak, A. Jungbauer, Irreversible and reversible impact on cellular behavior upon intra-experimental process parameter shifts in a CHO semi-continuous perfusion process, Biochem. Eng. J. 193 (2023).
[27] P. Satzer, D. Komuczki, M. Pappenreiter, A.L. Cataldo, B. Sissolak, A. Jungbauer, Impact of failure rates, lot definitions and scheduling of upstream processes on the productivity of continuous integrated bioprocesses, J. Chem. Technol. Biotechnol. 97 (2022) 2393–2403.
[28] M.N. São Pedro, T.C. Silva, R. Patil, M. Ottens, White paper on high-throughput process development for integrated continuous biomanufacturing, Biotechnol. Bioeng. 118 (2021) 3275–3286.
[29] T.C. Silva, M. Eppink, M. Ottens, Automation and miniaturization: enabling tools for fast, high-throughput process development in integrated continuous biomanufacturing, J. Chem. Technol. Biotechnol. 97 (2022) 2365–2375.
[30] I. Montes-Serrano, P. Satzer, A. Jungbauer, A. Dürauer, Characterization of hydrodynamics and volumetric power input in microtiter plates for the scale-up of downstream operations, Biotechnol. Bioeng. 119 (2022) 523–534.
[31] T.C. Silva, M. Eppink, M. Ottens, Small, smaller, smallest: Miniaturization of chromatographic process development, J. Chromatogr. A 1681 (2022).
[32] A. Nascimento, M.N. São Pedro, I.F. Pinto, M.R. Aires-Barros, A.M. Azevedo, Microfluidics as a high-throughput solution for chromatographic process development – The complexity of multimodal chromatography used as a proof of concept, J. Chromatogr. A 1658 (2021).
[33] M.N. São Pedro, M.H.M. Eppink, M. Ottens, Application of a fluorescent dye-based microfluidic sensor for real-time detection of mAb aggregates, Biotechnol. Prog. (2023).
[34] M.N. São Pedro, M. Isaksson, J. Gomis-Fons, M.H.M. Eppink, B. Nilsson, M. Ottens, Real-time detection of mAb aggregates in an integrated downstream process, Biotechnol. Bioeng. 120 (2023) 2989–3000.
[35] M.N. São Pedro, M.S. Santos, M.H.M. Eppink, M. Ottens, Design of a microfluidic mixer channel: First steps into creating a fluorescent dye-based biosensor for mAb aggregate detection, Biotechnol. J. 18 (2023).
[36] A. Javidanbardan, V. Chu, J.P. Conde, A.M. Azevedo, Microchromatography integrated with impedance sensor for bioprocess optimization: Experimental and numerical study of column efficiency for evaluation of scalability, J. Chromatogr. A 1661 (2022).
[37] M.A. Wahab, C. Domingues, A.M. Azevedo, V. Chu, J.P. Conde, M.R. Aires-Barros, An integrated microfluidic device for continuous bioprocessing, Sep. Purif. Technol. 332 (2024) 125702.
[38] M.D.C. Pons Royo, J.L. Beulay, E. Valery, A. Jungbauer, P. Satzer, Mode and dosage time in polyethylene glycol precipitation process influences protein precipitate size and filterability, Process Biochem. 114 (2022) 77–85.
[39] M.D.C. Pons Royo, I. Montes-Serrano, E. Valery, A. Jungbauer, P. Satzer, Milliscale reactors for integration of continuous precipitation and filtration, J. Chem. Technol. Biotechnol. 97 (2022) 3183–3192.
[40] A. Javidanbardan, A.M. Azevedo, V. Chu, J.P. Conde, A systematic approach for developing 3d high-quality pdms microfluidic chips based on micromilling technology, Micromachines 13 (2022).
[41] T.A. Barrett, A. Wu, H. Zhang, M.S. Levy, G.J. Lye, Microwell engineering characterization for mammalian cell culture process development, Biotechnol. Bioeng. 105 (2010) 260–275.
[42] A. Jungbauer, Continuous downstream processing of biopharmaceuticals, Trends Biotechnol. 31 (2013) 479–492.
[43] S. Malladi, M.J. Coolbaugh, C. Thomas, S. Krishnan, C.T. Varner, J. Walther, K. P. Brower, Design of a process development workflow and control strategy for single-pass tangential flow filtration and implementation for integrated and continuous biomanufacturing, J. Membr. Sci. 677 (2023).
[44] M. Krippl, T. Kargl, M. Duerkop, A. Dürauer, Hybrid modeling reduces experimental effort to predict performance of serial and parallel single-pass tangential flow filtration, Sep. Purif. Technol. 276 (2021).
[45] D. Fedorenko, A.K. Dutta, J. Tan, J. Walko, M. Brower, N.D.S. Pinto, A.L. Zydney, O. Shinkazh, Improved protein A resin for antibody capture in a continuous countercurrent tangential chromatography system, Biotechnol. Bioeng. 117 (2020) 646–653.
[46] J. Gomis-Fons, N. Andersson, B. Nilsson, Optimization study on periodic counter-current chromatography integrated in a monoclonal antibody downstream process, J. Chromatogr. A 1621 (2020).
[47] T.K. Kim, B. Sechi, J.J. Romero Conde, J. Angelo, X. Xu, S. Ghose, M. Morbidelli, M. Sponchioni, Design and economic investigation of a Multicolumn Countercurrent Solvent Gradient Purification unit for the separation of an industrially relevant PEGylated protein, J. Chromatogr. A 1681 (2022).
[48] A. Löfgren, J. Gomis-Fons, N. Andersson, B. Nilsson, L. Berghard, C. Lagerquist Hägglund, An integrated continuous downstream process with real-time control: A case study with periodic countercurrent chromatography and continuous virus inactivation, Biotechnol. Bioeng. 118 (2021) 1664–1676.
[49] C. Shi, Q.L. Zhang, B. Jiao, X.J. Chen, R. Chen, W. Gong, S.J. Yao, D.Q. Lin, Process development and optimization of continuous capture with three-column periodic counter-current chromatography, Biotechnol. Bioeng. 118 (2021) 3313–3322.
[50] Y.N. Sun, C. Shi, X.Z. Zhong, X.J. Chen, R. Chen, Q.L. Zhang, S.J. Yao, A. Jungbauer, D.Q. Lin, Model-based evaluation and model-free strategy for process development of three-column periodic counter-current chromatography, J. Chromatogr. A 1677 (2022).
[51] V. Warikoo, R. Godawat, K. Brower, S. Jain, D. Cummings, E. Simons, T. Johnson, J. Walther, M. Yu, B. Wright, J. McLarty, K.P. Karey, C. Hwang, W. Zhou, F. Riske, K. Konstantinov, Integrated continuous production of recombinant therapeutic proteins, Biotechnol. Bioeng. 109 (2012) 3018–3029.
[52] T. Eslami, L.A. Jakob, P. Satzer, G. Ebner, A. Jungbauer, N. Lingg, Productivity for free: Residence time gradients during loading increase dynamic binding capacity and productivity, Sep. Purif. Technol. 281 (2022).
[53] T. Eslami, M. Steinberger, C. Csizmazia, A. Jungbauer, N. Lingg, Online optimization of dynamic binding capacity and productivity by model predictive control, J. Chromatogr. A 1680 (2022).
[54] A.K. Dutta, J. Tan, B. Napadensky, A.L. Zydney, O. Shinkazh, Performance optimization of continuous countercurrent tangential chromatography for antibody capture, Biotechnol. Prog. 32 (2016) 430–439.
[55] B. Napadensky, O. Shinkazh, A. Teella, A.L. Zydney, Continuous countercurrent tangential chromatography for monoclonal antibody purification, Sep. Sci. Technol. (Philadelphia) 48 (2013) 1289–1297.
[56] R. Hahn, A. Jungbauer, Continuously operating separation unit, WO1995032781A1, (1995).
[57] Y. Guo, W. Ali, A. Schneider, A. Salma, T. Mayer-Gall, J.S. Gutmann, H. M. Fernandez Lahore, Megaporous monolithic adsorbents for bioproduct recovery as prepared on the basis of nonwoven fabrics, Electrophoresis 43 (2022) 1387–1398.
[58] Y. Guo, M. Kangwa, W. Ali, T. Mayer-Gall, J.S. Gutmann, C. Zenneck, M. Winter, A. Jungbauer, H.M. Fernandez Lahore, Moving adsorption belt system for continuous bioproduct recovery utilizing composite fibrous adsorbents, Front. Bioeng. Biotechnol. 11 (2023).
[59] L.M. Herlevi, M. Fernandez-Lahore, G. Ferreira, A fluidized-bed-riser adsorption system for continuous bioproduct recovery from crude feedstock, Biotechnol. Bioeng. 120 (2023) 2969–2976.
[60] D. Komuczki, N. Lingg, A. Jungbauer, P. Satzer, In-situ gradient formation by direct solid addition of buffer components, J. Chromatogr. A 1634 (2020).
[61] M. Isaksson, J. Gomis-Fons, N. Andersson, B. Nilsson, An automated buffer management system for small-scale continuous downstream bioprocessing, J. Chromatogr. A 1695 (2023).
[62] J. Scheffel, M. Isaksson, J. Gomis-Fons, H. Schwarz, N. Andersson, B. Norén, A. Solbrand, V. Chotteau, S. Hober, B. Nilsson, Design of an integrated continuous downstream process for acid-sensitive monoclonal antibodies based on a calcium-dependent Protein A ligand, J. Chromatogr. A 1664 (2022).
[63] H. Schwarz, J. Gomis-Fons, M. Isaksson, J. Scheffel, N. Andersson, A. Andersson, A. Castan, A. Solbrand, S. Hober, B. Nilsson, V. Chotteau, Integrated continuous biomanufacturing on pilot scale for acid-sensitive monoclonal antibodies, Biotechnol. Bioeng. 119 (2022) 2152–2166.
[64] C.S. Chen, K. Ando, N. Yoshimoto, S. Yamamoto, Linear flow-velocity gradient chromatography—An efficient method for increasing the process efficiency of batch and continuous capture chromatography of proteins, Biotechnol. Bioeng. 118 (2021) 1262–1272.
[65] A. Armstrong, K. Horry, T. Cui, M. Hulley, R. Turner, S.S. Farid, S. Goldrick, D. G. Bracewell, Advanced control strategies for bioprocess chromatography: Challenges and opportunities for intensified processes and next generation products, J. Chromatogr. A 1639 (2021).
[66] N. Lali, P. Satzer, A. Jungbauer, Residence time distribution in counter-current protein A affinity chromatography using an inert tracer, J. Chromatogr. A 1683 (2022).
[67] N. Andersson, J.G. Fons, M. Isaksson, S. Tallvod, D. Espinoza, L. Sjökvist, G. Z. Andersson, B. Nilsson, Methodology for fast development of digital solutions in integrated continuous downstream processing, Biotechnol. Bioeng. (2023).
[68] N. Lali, A. Jungbauer, P. Satzer, Traceability of products and guide for batch definition in integrated continuous biomanufacturing, J. Chem. Technol. Biotechnol. 97 (2022) 2386–2392.
[69] M.J. Coolbaugh, C.T. Varner, T.A. Vetter, E.K. Davenport, B. Bouchard, M. Fiadeiro, N. Tugcu, J. Walther, R. Patil, K. Brower, Pilot-scale demonstration of an end-to-end integrated and continuous biomanufacturing process, Biotechnol. Bioeng. 118 (2021) 3287–3301.
[70] F. Feidl, S. Vogg, M. Wolf, M. Podobnik, C. Ruggeri, N. Ulmer, R. Wälchli, J. Souquet, H. Broly, A. Butté, M. Morbidelli, Process-wide control and automation of an integrated continuous manufacturing platform for antibodies, Biotechnol. Bioeng. 117 (2020) 1367–1380.
[71] I. Ramos, N. Sharda, R. Villafana, K. Hill-Byrne, K. Cai, J. Pezzini, J. Coffman, Fully integrated downstream process to enable next-generation manufacturing, Biotechnol. Bioeng. 120 (2023) 1869–1881.
[72] F. Steinebach, N. Ulmer, M. Wolf, L. Decker, V. Schneider, R. Wälchli, D. Karst, J. Souquet, M. Morbidelli, Design and operation of a continuous integrated monoclonal antibody production process, Biotechnol. Prog. 33 (2017) 1303–1313.

[73] S. Vogg, M.K.F. Wolf, M. Morbidelli, Continuous and integrated expression and purification of recombinant antibodies, in, Methods Mol. Biol. (2018) 147–178.
[74] M.M. Rathore, S.A. Shah, D. Shukla, E. Bentafat, S. Bakiras, The role of AI, machine learning, and big data in digital twinning: a systematic literature review, challenges, and opportunities, IEEE Access 9 (2021) 32030–32052.
[75] A. Tiwari, V.S. Masampally, A. Agarwal, A.S. Rathore, Digital twin of a continuous chromatography process for mAb purification: Design and model-based control, Biotechnol. Bioeng. 120 (2023) 748–766.
[76] J. Coffman, M. Brower, L. Connell-Crowley, S. Deldari, S.S. Farid, B. Horowski, U. Patil, D. Pollard, M. Qadan, S. Rose, E. Schaefer, J. Shultz, A common framework for integrated and continuous biomanufacturing, Biotechnol. Bioeng. 118 (2021) 1721–1735.
[77] H. Mahal, H. Branton, S.S. Farid, End-to-end continuous bioprocessing: Impact on facility design, cost of goods, and cost of development for monoclonal antibodies, Biotechnol. Bioeng. 118 (2021) 3468–3485.
[78] J. Pollock, J. Coffman, S.V. Ho, S.S. Farid, Integrated continuous bioprocessing: Economic, operational, and environmental feasibility for clinical and commercial antibody manufacture, Biotechnol. Prog. 33 (2017) 854–866.