Received: 30 November 2020 | Revised: 15 January 2021 | Accepted: 12 March 2021

DOI: 10.1002/bit.27757

PERSPECTIVE

BIOTECHNOLOGY AND BIOENGINEERING WILEY

White paper on high-throughput process development for integrated continuous biomanufacturing

Mariana N. São Pedro[1] | Tiago C. Silva[1] | Rohan Patil[2] | Marcel Ottens[1]

[1]Department of Biotechnology, Delft University of Technology, Delft, The Netherlands

[2]Global CMC Development, Sanofi, Framingham, Massachusetts, USA

Correspondence

Marcel Ottens, Department of Biotechnology, Delft University of Technology, van der Maasweg 9, Delft, 2629 HZ, The Netherlands.
Email: m.ottens@tudelft.nl

Funding information

EU H2020 MCS ITN, Grant/Award Number: 812909 CODOBIO

Abstract

Continuous manufacturing is an indicator of a maturing industry, as can be seen by the example of the petrochemical industry. Patent expiry promotes a price competition between manufacturing companies, and more efficient and cheaper processes are needed to achieve lower production costs. Over the last decade, continuous biomanufacturing has had significant breakthroughs, with regulatory agencies encouraging the industry to implement this processing mode. Process development is resource and time consuming and, although it is increasingly becoming less expensive and faster through high-throughput process development (HTPD) implementation, reliable HTPD technology for integrated and continuous biomanufacturing is still lacking and is considered to be an emerging field. Therefore, this paper aims to illustrate the major gaps in HTPD and to discuss the major needs and possible solutions to achieve an end-to-end Integrated Continuous Biomanufacturing, as discussed in the context of the 2019 Integrated Continuous Biomanufacturing conference. The current HTPD state-of-the-art for several unit operations is discussed, as well as the emerging technologies which will expedite a shift to continuous biomanufacturing.

KEYWORDS

high-throughput process development, integrated continuous biomanufacturing, microfluidics, modeling, process analytical technology

1 | INTRODUCTION

A possible solution to establish more efficient and flexible processes in the biopharmaceutical industry is to transition to continuous integrated manufacturing: An improvement in productivity, product quality and consistency can be achieved while drastically reducing the facility footprint and manufacturing costs (Rathore et al., 2015; Shukla et al., 2017; Somasundaram et al., 2018). Continuous bioprocessing utilizes a continuous flow of material through the various unit operations such that, at steady state, product of consistent quality is being produced as long as the operation runs (Rathore et al., 2015).

Many manufacturing sectors, such as chemical, food, and pharmaceutical have long adopted continuous manufacturing, but its

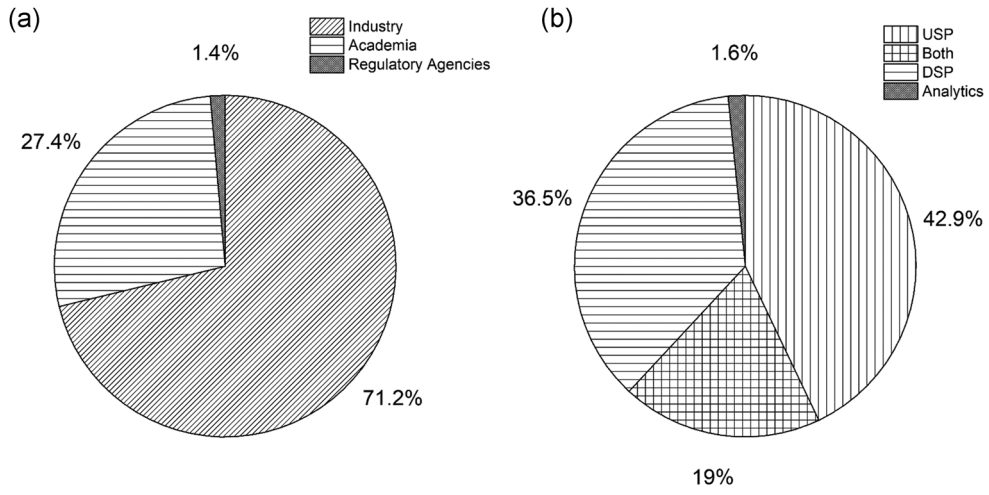
Abbreviations: CQA, critical quality attribute; HTPD, high-throughput process development; HTS, high-throughput screening; ICB, integrated continuous biomanufacturing; LC, liquid chromatography; MM, mechanistic models; PAT, process analytical technology; QbD, quality by design.

Mariana N. São Pedro and Tiago C. Silva contributed equally to this study.

implementation in biotechnology manufacturing, particularly of biotherapeutics, is still behind (Rathore et al., 2015; Zydney, 2016). However, Walther et al. (2015) conducted an economic analysis into an integrated continuous biomanufacturing platform and concluded that it would allow to reduce costs by 55% relative to conventional batch processing, demonstrating the promise of implementing a continuous bioprocess for the manufacturing of recombinant proteins. Therefore, there is a growing interest, from both academia and industrial research, to develop continuous processing systems (Konstantinov & Cooney, 2015). An example is the ongoing project Continuous Downstream Processing of Biologics—CODOBIO (2018—2022), a research program with the main goal of facing the future transition challenges to a continuous downstream process, implementing innovative integrated continuous manufacturing in the bioindustry.

The Integrated Continuous Biomanufacturing (ICB) conference aims to bring together academia and industry peers to shed some light on the recent advances and discoveries on bioprocesses, which could help to achieve the "holy grail" of a continuous end-to-end process and real-time release. Within the fourth edition of the conference (ICB IV), held in Cape Cod (Massachusetts, USA) in 2019, a workshop entitled "High-Throughput Methodologies for ICB" brought together participants with different backgrounds (Figure 1). The workshop aimed to trigger the discussion on which are the perceived gaps in high-throughput (HT) technologies for process development, what are the current needs for ICB, the major problems and the correspondent expected solutions, and what is currently being done in research to achieve this continuous biomanufacturing. With a total of 73 participants (from which the vast majority belonging to industry), the workshop aimed to collect relevant and up-to-date data on what is the view regarding the shift to continuous manufacturing in the biopharmaceutical realm, and what still needs to be done to put this industry closer to this objective. The attendees were asked to split and mix with their peers from different backgrounds and affiliations. This aimed to achieve a more diverse discussion between the groups and promote a greater need for consensus when posting an answer. Overall, six out of the eight groups were mixed in terms of affiliation and a good mix of backgrounds was also possible to achieve (Figure 1c).

Although current trends in the production of biopharmaceuticals are to gradually move from batch processes to integrated continuous processing strategies, to perform the process in a continuous mode, an integration of the different unit operations in one single production and purification train is the ultimate goal, adding to each unit operation the capacity of recycling streams and the ability to purge impurities as required by the process (Rathore et al., 2015). Furthermore, analytical techniques must provide real-time information of each biomanufacturing step to gain knowledge and control over the overall process.



(c)

Groups	Industry	Academia	Regulatory Agencies	USP	DSP	Process Development	Analytics
1	6	4	-	5	2	2	1
2	6	3	1	2	4	3	-
3	4	5	-	*information not collected			
4	9	-	-	4	3	2	-
5	6	3	-	5	4	-	-
6	6	2	-	2	3	3	-
7	10	-	-	5	3	2	-
8	5	3	-	4	4	-	-

FIGURE 1 Workshop participants background: (a) Area where the participants work in: Industry, academia, or regulatory agencies; (b) Function/Department where the participants work in: USP, upstream processing; DSP, downstream processing; process development, which implicates both USP and DSP function; and analytics. (c) Descriptive constitution of each of the eight groups formed during the workshop, according to the area/function of each participant

Therefore, this white paper will discuss the major gaps in HTPD for the different unit operations, the integration problems present in the continuous manufacturing of biopharmaceuticals, and the already available tools to overcome these challenges. By covering what is the state-of-the-art for several established technologies, the paper aims to shed some light on the emerging tools for process development to enable and accelerate the shift to a continuous process.

2 | OUTCOME OF THE WORKSHOP

After identifying the state-of-the-art in HTPD, it was possible to pinpoint the gaps in HTPD for continuous biomanufacturing. In Figure 2, the unit operations/system components perceived by the participants as having a major gap for HTPD are presented: Cell culture was unanimously identified by every group, followed by the filtration unit operation and the current analytical tools for a continuous process. Some groups also pointed out potential gaps regarding cell media development, viral inactivation, chromatography, and other unit operations, such as centrifugation and aqueous two-phase extraction.

Cell culture has had the industry's attention for several years, with higher titer-producing strains being developed. There is already equipment available for the HTPD of cell culture, still, such systems come at a high price, as will be discussed further, which makes it to be perceived by all groups as being an area where a significant gap is present.

On the contrary, filtration has been an overlooked unit operation when it comes to HTPD. When many researchers focused efforts on the optimization of chromatography, most likely due to being one of the most expensive unit operations, filtration steps have stayed behind when it comes to HT alternatives. Although a batch filtration step can easily be implemented in a continuous process, the optimization of such steps can become costly, as scale-down models are lacking, with a large investment to find optimal operating conditions needed. Therefore, there usually exists a compromise between oversizing the equipment or spending a considerable amount for the optimization of this unit operation. However, recent studies developed by Fernandez-Cerezo et al. (2019) used a downscale method for a filtration unit resorting to using a combination of critical flow regime analysis, modeling, and experimentation to predict the performance of a pilot-scale system, therefore, showing a possible HT tool for future conditions studies in this unit operation.

Regarding the analytical tools needed for implementation in a continuous process, the major gaps indicated are related to the quantity of sample and different techniques necessary to obtain information on the overall process. Furthermore, there are still great limitations in the available HT analytical tools used in ICB, highlighted by the limited number of techniques which were able to be integrated with HT platforms. A possible explanation for this limitation is related to the lengthy analysis times of each technique, which can make it difficult to employ for process monitoring and control. The current trend to tackle these analytical shortcomings is the creation of at-line sensors, which can provide real-time measurement and data on a continuous process, titled process analytical techniques (PAT), and will be further discussed in this paper.

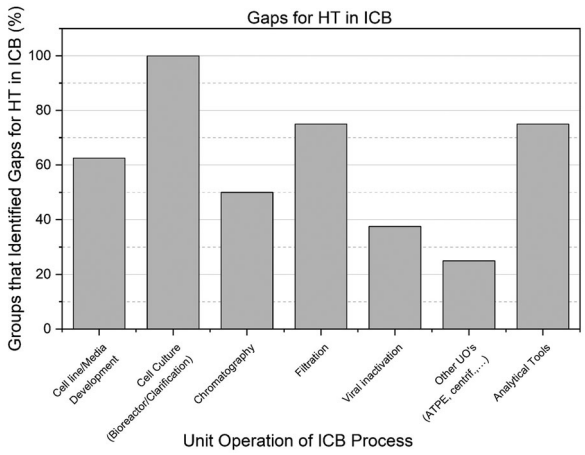


FIGURE 2 Major gaps indicated for high-throughput (HT) development in integrated continuous biomanufacturing (ICB) by the participants in the workshop

3 | STATE-OF-THE-ART IN CONTINUOUS BIOMANUFACTURING

The workshop participants were tasked to come up with HT technologies that are currently in use in process development for continuous biomanufacturing. Although some unit operations got more attention than others, the main goal of this activity was to understand what are the mainly used equipment and tools involved in the development of processes for the desired successful shift to a continuous operation. The main tools already in place are the automated systems for liquid-handling, where there is the possibility to use tailored equipment for a specific unit operation or an equipment with a broader capability that can have minor adaptions for different uses. In Table 1, the state-of-the-art of HTPD tools for the development of different unit operations is summarized.

The use of liquid-handling stations for the determination of best-operating conditions for cell media development and antibody purification has gained popularity over the past years (Treier et al., 2012). The work developed by Schmidt et al. (2016) shows an improvement of previous studies where a platform for the purification of an antibody in an automated two-step chromatography purification was developed. The HT system showed limitations in the flow rate that could be used in the RoboColumns, which affected the value for the dynamic binding capacity that could be obtained, but the results were comparable to the previously used ÄKTA™ systems. This platform process allows for the purification of hundreds of monoclonal antibodies per week, even at low feed concentrations.

In terms of available HTPD tools for viral inactivation and viral clearance, even though the participants indicated to be a

TABLE 1 State-of-the-art in the integrated continuous biomanufacturing (ICB) field

Technology	Answers	References from literature
Cell line/media development	Ambr® 15/250/P	Sandner et al. (2019); Xu et al. (2017)
	Liquid handling systems (Tecan)	Shi et al. (2011)
	Beacon®	Le et al. (2020)
	Spin tubes	Strnad et al. (2010)
Cell culture (bioreactor)	Ambr® 15/250/P	Sandner et al. (2019); Xu et al. (2017)
	Small scale bioreactors	Schwarz et al. (2020)
Cell culture (clarification)[a]	Pendotech	Fedorenko et al. (2020); N. D. S. Pinto et al. (2020)
	Filtration skids	Arunkumar et al. (2017)
	Acoustic wave	Baptista et al. (2013); Granicher et al. (2020)
	ATF/TFF	Arunkumar et al. (2017); N. D. S. Pinto et al. (2020)
	Centrifugation	Hogwood et al. (2013); Tait et al. (2009)
Chromatography	Tecan	McDonald et al. (2016); Sisodiya et al. (2012)
	Predictor plates	Bergander et al. (2008)
	RoboColumns	Keller et al. (2015); Schmidt et al. (2016)
	Mechanistic understanding using HT	Kumar et al. (2015); Nfor et al. (2012); Pirrung et al. (2018)
	ÄKTA™	Keller et al. (2015)
	Multicolumn chromatography (MCC)	Gjoka et al. (2015)
Filtration[ab]	SPTFF	Clutterbuck et al. (2017); Fernandez-Cerezo et al. (2019)
	UF membranes	Baek et al. (2017)
	96-well plate	Tang et al. (2020)
Viral inactivation[ab]	Low pH/mixing	David et al. (2019); Gillespie et al. (2019) Orozco et al. (2017); Parker et al. (2018)
	Solvents/detergents	Lofgren et al. (2020); Martins et al. (2019)
	Filters	Lute et al. (2020); Tang et al. (2020)
	Temperature	Gillespie et al. (2019)
	Purification steps	Gjoka et al. (2017)
	Tubular reactor	Gillespie et al. (2019); Orozco et al. (2017); Parker et al. (2018)
	Two chambers (not continuous)	Gjoka et al. (2017)
Analytical tools[ab]	UV	Kamga et al. (2013); S. W. Li et al. (2014)
	pH	Gillespie et al. (2019); Zelger et al. (2016)
	Conductivity	Zelger et al. (2016)
	Raman spectroscopy	Kornecki & Strube (2018); M. Y. Li et al. (2018); Nagy et al. (2017)
	NIR/MIR spectroscopy	Capito et al. (2013); Capito et al. (2015); Thakur, Hebbi, et al. (2020); Thakur, Thori, et al. (2020)
	MALS	Patel et al. (2018); Sauer et al. (2019)
	Online LC	Rathore, Wood, et al. (2008); Rathore, Yu, et al. (2008); Sharma et al. (2006)
	Mass spectrometry	Dong et al. (2016); Liu et al. (2020); Steinhoff et al. (2016)

Abbreviations: HT, high-throughput; LC, liquid chromatography; MIR, mid-infrared; NIR, near-infrared; STPFF, single-pass tangential flow filtration.

[a]Mainly a description of what is being done in the scope of ICB and not completely related to HT.

[b]Few groups answered this question: Either they had some struggles to find an answer or didn't consider this technology to be a bottleneck.

considerable gap in development, recent studies have been published demonstrating the potential of developing a virus filter micro-scale HTPD model. Tang et al. (2020) used, in combination with an automated liquid handling system, a 96-well filter plate to assess its suitability to be a novel microscale HTPD scaledown model. With these types of tools, HT virus filtration optimization is now an option, enabling rapid process development for continuous biomanufacturing. Additionally, to make this important step continuous, several lab-scale models of viral inactivation system have been simulated, designed, and built: for example, Gillespie et al. (2019) tested multiple incubation chamber designs to allow narrow residence time distributions; whereas Parker et al. (2018) used a comprehensive computational fluid dynamics model to create a laminar flow tubular reactor.

4 | CURRENT NEEDS IN THE INTEGRATED CONTINUOUS BIOPROCESSING

From the information gathered during the workshop, the major challenges pointed out by the participants in HTPD for the implementation of continuous bioprocessing are presented in Table 2. Furthermore, it was requested to the participants to propose possible solutions for each of the challenges discussed. Modeling and micro/nanofluidics were the main suggestions for the fields to further invest/prioritize to make an easier and smoother transition to continuous biomanufacturing. These proposed solutions and other current needs in ICB will be further discussed, with a particular focus given to PAT tools and unit operation connection.

4.1 | Process analytical technique

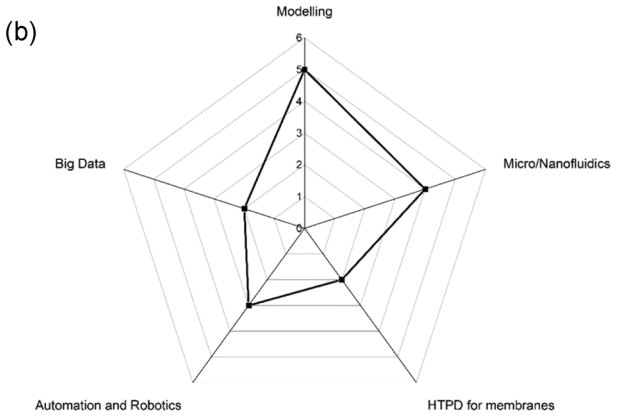
PAT was defined as "a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality" (U.S. Department of Health and Human Services, 2004). The ultimate goal of implementing PAT in the biopharmaceutical industry is to design and develop well-understood processes that will reliably ensure a predefined quality in the final product by either monitoring the raw material or in-process product attributes in real-time to control the process, the critical quality attributes (CQAs) (Glassey et al., 2011; Read et al., 2010). The clear

TABLE 2 (a) Major problems indicated by the participants of the workshop, with the proposed solutions/fields to invest/prioritize for ICB process development; (b) Summary of the suggested tools by the participants (only six groups answered this question) as solutions for current gaps/problems with HTPD in ICB

(a)

Major Problem	Suggested Solution
Scale Down of Filtration Processes	Microfluidics
USP-DSP Integration	Microfluidics Modelling
Low-Throughput Cell Line and Media Development	Integrate robotics into existing systems
Cell Culture Development (bioreactor/cell retention device) is not mechanistic	Big Data Analysis / Smart HTPD (Artificial Intelligence / Mechanistic Modelling)
Process Development for Cell Culture	Microfluidics
Scability of Mini-Columns (done in batch mode)	Modelling
Integration of Chromatographic Steps	Modelling

(b)



Abbreviations: DSP, downstream processing; HTPD, high-throughput process development; ICB, integrated continuous biomanufacturing; USP, upstream processing.

process control and understanding provided by the PAT framework supports as well the quality by design (QbD) approach adopted by the biopharmaceutical industry. QbD was defined as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management" (Wasalathanthri et al., 2020). Hence, PAT implementation will aid this systematic process development method, QbD, by providing a better understanding of the products and design processes that will ensure consistent product quality.

The crucial element for PAT applications in a continuous process is to be able to gather CQA information for the process and elicit a timely response to facilitate control. It is necessary for the analytical measurements to be available in the time-frame necessary to facilitate real-time decision making (Read et al., 2010). Additionally, to easily implement a PAT tool, the cost required for the instrumentation should not be high, at least until it does not drastically increase the biopharmaceutical production cost. Furthermore, the chosen analytical technique has to be precise, accurate, and robust (Mandenius & Gustavsson, 2015). Although a continuous process has a lot of gain from PAT implementation, these types of tools have been fairly unexplored (Read et al., 2010; Sharma et al., 2006) and for the advancement of continuous processes, important improvements in sensor technology, configuration, and robustness are still required (Fisher et al., 2019).

Regarding upstream processing, classical process sensors provide information on process variables such as temperature, pH, dissolved gases, and foam levels (Fisher et al., 2019). However, more robust techniques, involving spectrometric sensors, have been successfully implemented for process monitoring. For example, near-infrared spectroscopy has been most extensively studied to determine the concentration of individual components in the cell culture broth, as demonstrated by Arnold et al. (2002). Furthermore, Raman spectroscopy can be used not only to analyze broth component profiles as well as to monitor structural/chemical changes in proteins, of particular interest to on-line monitor aggregate formation (Gryniewicz & Kauffman, 2008) or quality attributes such as glycosylation (M. Y. Li et al., 2018). Recently, liquid chromatography-mass spectrometry-based multiattribute methods have emerged as an important PAT, allowing for simultaneous monitoring of the product quality attributes such as glycan profile, charge variants, and purity of biotherapeutics (Wasalathanthri et al., 2020). By developing a platform with the collection of cell-free samples from bioreactors, followed by automated HT purification using an automated liquid handling system, Dong et al. (2016) demonstrated that it was possible to produce a "near-real-time" measurement, laying out a solid foundation towards using this technique to monitor multiple CQAs during the entire biomanufacturing process.

For the downstream processing, PAT implementation is still fairly limited due to, in part, a lack of sensor options: pH, conductivity, absorbance, and pressure sensors do not actually measure quality attributes of the biomolecule, such as protein aggregation. However, Kamga et al. (2013) employed multiwave UV spectra to effectively determine the concentration of individual components in a protein mixture, accurately predicting aggregate concentration relative to the protein of interest. Additionally, for the chromatography steps, implementing PAT can be challenging because of the typical short process times of these unit operations (Read et al., 2010). Sharma et al. (2006) demonstrated that, with on-line analytical LC, continuous monitoring of the chromatography step for aggregate peaks can be achieved. An on-line high-performance liquid chromatography (HPLC) system was used to investigate the real-time pooling of a process chromatography column and it was programmed to stop collecting when the aggregate peak starts, showing the feasibility of using PAT to facilitate real-time decisions for column pooling based on product quality attributes.

Therefore, in a continuous process, a PAT tool must provide decisive information for subsequent process steps on-line, making continuity of processing possible (Mandenius & Gustavsson, 2015). In the future, the development and implementation of these PAT will allow for the design of a manufacturing process that will deliver a consistent, well-defined quality product and improve process efficiency. Foreseeable challenges include implementing noninvasive process monitoring techniques and incorporating advanced sensors into automated process control strategies (Fisher et al., 2019).

4.2 | Data collection/modeling

The biological processes in the biotechnology industry present many challenges and are usually, if not always, less straightforward than in other industries. The complexity of the operations, especially fermentation, drove the industry to a trial-and-error mode of optimization for years. However, in recent years with the application of QbD and PAT initiatives (Rathore, 2009; U.S. Department of Health and Human Services, 2011), there has been a greater push for a better understanding of the process. This has empowered scientists and engineers to have greater knowledge and details of each operation and not treat processes purely as black boxes.

The ability to translate a process, whether it is a relatively complex process, such as a fermentation, or a simpler process, such as a mixing tank, into a mathematical model has not only allowed a greater process understanding but also a reduction in time and experiments needed for optimization (Chhatre et al., 2011; Nfor et al., 2013). Mechanistic models (MM) aim to accurately describe the physicochemical phenomena of the system to be described, and several examples of such models have been published for bioprocesses (Hebbi et al., 2020; Kumar et al., 2015; Nfor et al., 2012; Yahia et al., 2015). Besides models that are purely mechanistic, hybrid approaches using MM and machine learning, like Artificial Neural Networks, can help to ease up the computational load on the computer, for example, by using data sets for the determination of certain parameters and then use these as input to the models. This accelerates the process development and provides faster results, as has been shown in literature, for estimation of process parameters (Wang et al., 2017) and optimization of full downstream

processes (Pirrung et al., 2017). Once such models are tuned and trained, the output of these computations will provide valuable insight into the processes. It is clear that models are of great importance for the leap into integrated continuous biomanufacturing, both in the process development stage and also once such processes are implemented in the production facilities. Modeling cannot, however, be completely detached from the experimental work and data. It needs data to estimate parameters, to train models, and ultimately, to validate them. Moreover, mathematical models are important for the implementation and realization of much-needed control strategies, which are crucial for ensuring the proper functioning of such a complex production train.

The use of models is now widely accepted by industry and is certainly a critical feature of future continuous processes. The ability to make decisions on the fly depending on unexpected changes to the process based on an accurately described model is something the industry requires. This also raises the need for reliable and accurate data collection. Coupled to increasingly improved sensors, there is a great need to have very fast and accurate analytics to not only collect data on the process's behavior in order for fast action to take place, but also to be able to monitor and control the CQAs and maintain the final product quality. Considering all the unit operations and processes taking place in a production facility, the amount of data generated at once can be overwhelming. Though this generation of large amounts of data is of paramount importance for the process understanding and monitoring, automation of the analyses of the data is crucial (Oliveira, 2019). The integrated continuous biomanufacturing initiatives are longing for ways to accommodate and make good use of all the generated data, whether it is destined to process control, process overview, or process development.

4.3 | Upstream/downstream processing connection (and unit operations)

For a truly integrated continuous biomanufacturing, the uninterrupted connection of continuous unit operations (upstream and downstream) is necessary, with no or minimal isolated intermediate or hold steps occurring between them.

Several examples of integrating a continuous upstream process with immediate capture have been established (Kamga et al., 2018; Karst et al., 2017), with the use of perfusion culture to continuously remove media and extracellular material from the bioreactor. A major challenge with integrating both processes is synchronizing the upstream perfusion flow rate with the downstream purification flow rate (Fisher et al., 2019). Synchronized control systems between upstream and downstream systems are also lacking. Therefore, a deviation in the upstream process will not be detected by downstream systems (feedforward control) or vice versa (feedback control). This type of system needs to be developed and implemented since several upstream parameters can impact subsequent downstream operations. Karst et al. (2017) demonstrated the possibility of implementing feedback control with the installation of an at-line HPLC to provide titer data on bioreactor harvest to modulate the operating conditions of the capture step and regulate the continuous volumetric flow rate by using control loops.

Though continuous upstream bioprocessing is reasonably well established, the integration of a full continuous downstream processing is still a developing field. For continuous capture and polishing chromatography, two main systems can be applied: Periodic counter-current chromatography (PCC) and simulated moving bed chromatography. In a truly integrated continuous chromatography platform, process synchronization can be achieved by enforcing the residence time in a column to exceed the successive column steps. To ensure that poor quality eluent material from one column is not pooled with material to the next functioning column, real-time monitoring and feedback control are necessary. The pooling between columns might also introduce the risk of cross-contamination, which this feedback control strategy might be able to detect and divert the effluent away from the second column (Fisher et al., 2019). At a small scale, the connection between different chromatography columns and an ultrafiltration unit for the purification of a recombinant protein was developed by Gomis-Fons et al. (2019). An external controller, Orbit, was used to make the system automated and open and closed-loop control strategies were applied: UV was monitored in-line and used for automatic product pooling based on cut-off absorbance levels, for example. Furthermore, in an integrated continuous downstream process, a significant reduction in consumable needs, such as chromatography media and buffer consumption, will lead to a drastic reduction in operating and costs. Gjoka et al. (2017) converted four purification unit operations into a continuous process, reducing the resin volume and buffer required by more than 95% and 44% compared to the corresponding batch process, respectively, and significantly decreasing consumables consumption.

Therefore, a fully integrated continuous process has the potential to improve quality, cost, speed, and flexibility, with the most urgent challenge to be tackled being the creation of a global monitoring and control strategy for the entire biomanufacturing process. This would entail not only the monitoring and control of continuous measurements at all inlet and outlet streams (PAT framework) but also realistic feedback and feedforward control strategy to ensure the final product quality. Thus far, to the author's knowledge, complete end-to-end integration in manufacturing processes has still to be reported. However, Godawat et al. (2015) were able to combine a perfusion bioreactor with two periodic PCC units for initial capture and successive ion-exchange steps, showing it is feasible to fully create and integrate an end-to-end continuous bioprocessing platform. More recently, Coolbaugh et al. (2021) have demonstrated such end-to-end continuous processes are scalable by showing a successful proof-of-concept at pilot-scale.

4.4 | Other needs

The aforementioned needs represent three big realms where further development is needed. However, there are also some needs that are

missing and others that despite not being totally missing still lack the practicality and/or affordability to be reliable solutions. The increased democratization of high-throughput screening (HTS) has led facilities around the world to more automated labs and miniaturized assays.

The use of automated liquid-handling systems has long been established as the standard for HTPD in downstream (mainly chromatography), as methods for the determination of adsorption isotherms and even full chromatographic runs have been described (Evans et al., 2017; Kiesewetter et al., 2016; Nfor et al., 2010; Wiendahl et al., 2008). The use of such equipment allows for the automation of the assays while keeping the used volumes low, yielding a faster and more cost-effective analysis. For upstream, there have been solutions for HTPD, however, these usually come with a very high price tag such as the Ambr® systems (Xu et al., 2017), which can discourage scientists and companies from investing. The industry is, therefore, calling for affordable alternatives and sees in microfluidics a good opportunity to fill this need. When it comes to cell line development, the current state-of-the-art for companies without the Ambr® system is to take the better-performing strains in batch mode and then test it in perfusion mode. There is, therefore, a need for a deeper understanding of cell biology which will ultimately lead to the development of better cell lines at affordable prices, and microfluidics steps up to offer that (Kwon et al., 2017).

Microfluidics has already shown to be a powerful scale-down model of equipment capable of mimicking several unit operations with the advantage of using less sample volume and achieving faster assays. These devices are still paving their way into the repertoire of process development but have already shown promising results for different unit operations such as crystallization (L. Li & Ismagilov, 2010), chromatography (I. F. Pinto et al., 2018), cell culture (Mehling & Tay, 2014), aqueous two-phase systems (Silva et al., 2014), biocatalysis (Zhu et al., 2020) and as a promising scale-down model for HTS equipment, where parallel assays at a manifold volume and time reduction have been previously demonstrated (Rho et al., 2017). However, filtration has lacked a scale-down model that would allow for HTPD of the specific unit operations. Membrane filtration is also not widely used in microfluidics, with both inertial and membrane filtration being reported as alternatives (Bhagat et al., 2008; Chen & Shen, 2017). The adaption of liquid-handling stations to the HTPD of such unit operation is still in very early stages. Filtration process development usually needs a large amount of materials and time-consuming work. The use of HTS equipment for such a system emphasizes on reducing reagent consumption in process development while avoiding the oversizing of equipment, the consequence of a poorer process knowledge (Tang et al., 2020).

5 | CONCLUSION

The change to continuous processing is a natural path for a maturing industry, and biopharmaceutical industries are following it, with technological advancements empowering this shift more and more. The advantages of this technology are great and well demonstrated, and it has been evidenced that it allows for process cost reductions at different scales, even when compared to the most established batch processing modes and different production scales (Hummel et al., 2019).

Continuous processing allows, in general, for more efficient processes while reducing the footprint. Increasing the volumetric flow translates into a smaller increase in equipment and consumables cost for continuous processing than for what is observed for batch processes, due to more efficient use of equipment. The counterpart of continuous bioprocessing is the increased need for fast analytics and control, that can provide real-time responses for fluctuations in operational conditions to guarantee product quality.

Although the technological breakthroughs have been immense over the past 20 years, we can understand that academia and industry are eager for better processing technologies. From the workshop outcome it is possible to conclude that although there are plenty of options for process development and optimization, the room for improvement is still quite large, either to have new technologies or to find a way to cut down the prices of existing technologies to democratize process development. Among the tools perceived as the most promising to fulfill current gaps in ICB are modeling and micro/nanofluidics. This goes in accordance with the current demands of regulatory agencies translated in PAT and QbD initiatives, where a higher process understanding is in order and control of the final product quality is achieved, reducing the product variance in meeting CQA's.

Recent advances in both upstream and downstream processing research allowed to achieve competitive unit operations running in continuous mode, allowing these new processes to outperform the previously established ones. As the upstream and downstream processing have been developed separately throughout the years, the challenge now relies on integrating all these continuous unit operations into a continuous end-to-end manufacturing process (Gronemeyer et al., 2017). The integration of software and hardware is important to achieve a fully continuous process, as well as process control, both feedforward and feedback, so that faster decisions are made according to what is happening in other unit operations. The further development of PAT and a synchronization of control systems will be the key enablers of the shift to an end-to-end continuous process in the biopharmaceutical industry (Fisher et al., 2019).

Reducing the time to market usually hinders the implementation of a continuous process, as it is easier to “play safe” and assure that the “race is won.” Biosimilars can, however, take advantage of the patent expiry and bet on such processing mode, aiming to achieve a more efficient and less expensive process allowing the biosimilars producing companies to compete with major players.

ACKNOWLEDGMENTS

The authors wish to thank the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 812909 CODOBIO, within the Marie Skłodowska-Curie European Training Networks framework.

The authors also wish to thank the 2019 ICB conference chairs for providing the opportunity to conduct this workshop and get valuable feedback from participants.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Mariana N. São Pedro and Tiago Castanheira Silva equally contributed with the following: Gathering and treatment of the data and feedback obtained from the workshop participants and writing of the original draft of the manuscript. Rohan Patil and Marcel Ottens conceived, organized, and conducted the workshop, as well as reviewing and editing this article.

REFERENCES

Arnold, S. A., Gaensakoo, R., Harvey, L. M., & McNeil, B. (2002). Use of at-line and in-situ near-infrared spectroscopy to monitor biomass in an industrial fed-batch *Escherichia coli* process. *Biotechnology and Bioengineering*, *80*(4), 405–413. https://doi.org/10.1002/bit.10383

Arunkumar, A., Singh, N., Peck, M., Borys, M. C., & Li, Z. J. (2017). Investigation of single-pass tangential flow filtration (SPTFF) as an inline concentration step for cell culture harvest. *Journal of Membrane Science*, *524*, 20–32. https://doi.org/10.1016/j.memsci.2016.11.007

Baek, Y., Singh, N., Arunkumar, A., Borys, M., Li, Z. J., & Zydney, A. L. (2017). Ultrafiltration behavior of monoclonal antibodies and Fc-fusion proteins: Effects of physical properties. *Biotechnology and Bioengineering*, *114*(9), 2057–2065.

Baptista, R. P., Fluri, D. A., & Zandstra, P. W. (2013). High-density continuous production of murine pluripotent cells in an acoustic perfused bioreactor at different oxygen concentrations. *Biotechnology and Bioengineering*, *110*(2), 648–655. https://doi.org/10.1002/bit.24717

Bergander, T., Nilsson-Välimaa, K., Öberg, K., & Lacki, K. M. (2008). High-throughput process development: Determination of dynamic binding capacity using microtiter filter plates filled with chromatography resin. *Biotechnology Progress*, *24*(3), 632–639.

Bhagat, A. A. S., Kuntaegowdanahalli, S. S., & Papautsky, I. (2008). Inertial microfluidics for continuous particle filtration and extraction. *Microfluidics and Nanofluidics*, *7*(2), 217–226. https://doi.org/10.1007/s10404-008-0377-2

Capito, F., Skudas, R., Kolmar, H., & Stanislawski, B. (2013). Host cell protein quantification by Fourier transform mid-infrared spectroscopy (FT-MIR). *Biotechnology and Bioengineering*, *110*(1), 252–259. https://doi.org/10.1002/bit.24611

Capito, F., Zimmer, A., & Skudas, R. (2015). Mid-infrared spectroscopy-based analysis of mammalian cell culture parameters. *Biotechnology Progress*, *31*(2), 578–584. https://doi.org/10.1002/btpr.2026

Chen, X., & Shen, J. (2017). Review of membranes in microfluidics. *Journal of Chemical Technology & Biotechnology*, *92*(2), 271–282. https://doi.org/10.1002/jctb.5105

Chhatre, S., Farid, S. S., Coffman, J., Bird, P., Newcombe, A. R., & Titchener-Hooker, N. J. (2011). How implementation of quality by design and advances in biochemical engineering are enabling efficient bioprocess development and manufacture. *Journal of Chemical Technology & Biotechnology*, *86*(9), 1125–1129.

Clutterbuck, A., Beckett, P., Lorenzi, R., Sengler, F., Bisschop, T., & Haas, J. (2017). Single-pass tangential flow filtration (SPTFF) in continuous biomanufacturing. *Continuous Biomanufacturing: Innovative Technologies and Methods*, 423–456.

CODOBIO. (2018–2022). Continuous downstream processing of biologics. https://www.codobio.eu/

Coolbaugh, M. J., Varner, C. T., Vetter, T. A., Davenport, E. K., Bouchard, B., Fiadeiro, M., Tugcu, N., Walther, J., Patil, R., & Brower, K. (2021). Pilot-scale demonstration of an end-to-end integrated and continuous biomanufacturing process. *Biotechnology and Bioengineering*. https://doi.org/10.1002/bit.27670

David, L., Maiser, B., Lobedann, M., Schwan, P., Lasse, M., Ruppach, H., & Schembecker, G. (2019). Virus study for continuous low pH viral inactivation inside a coiled flow inverter. *Biotechnology and Bioengineering*, *116*(4), 857–869. https://doi.org/10.1002/bit.26872

Dong, J., Migliore, N., Mehrman, S. J., Cunningham, J., Lewis, M. J., & Hu, P. (2016). High-throughput, automated protein A purification platform with multiattribute LC-MS analysis for advanced cell culture process monitoring. *Analytical Chemistry*, *88*(17), 8673–8679. https://doi.org/10.1021/acs.analchem.6b01956

Evans, S. T., Stewart, K. D., Afdahl, C., Patel, R., & Newell, K. J. (2017). Optimization of a micro-scale, high throughput process development tool and the demonstration of comparable process performance and product quality with biopharmaceutical manufacturing processes. *Journal of Chromatography A*, *1506*, 73–81.

Fedorenko, D., Dutta, A. K., Tan, J., Walko, J., Brower, M., Pinto, N. D. S., Zydney, A. L., & Shinkazh, O. (2020). Improved protein A resin for antibody capture in a continuous countercurrent tangential chromatography system. *Biotechnology and Bioengineering*, *117*(3), 646–653. https://doi.org/10.1002/bit.27232

Fernandez-Cerezo, L., Rayat, A., Chatel, A., Pollard, J. M., Lye, G. J., & Hoare, M. (2019). An ultra scale-down method to investigate monoclonal antibody processing during tangential flow filtration using ultrafiltration membranes. *Biotechnology and Bioengineering*, *116*(3), 581–590. https://doi.org/10.1002/bit.26859

Fisher, A. C., Kamga, M. H., Agarabi, C., Brorson, K., Lee, S. L., & Yoon, S. (2019). The current scientific and regulatory landscape in advancing integrated continuous biopharmaceutical manufacturing. *Trends in Biotechnology*, *37*(3), 253–267. https://doi.org/10.1016/j.tibtech.2018.08.008

Gillespie, C., Holstein, M., Mullin, L., Cotoni, K., Tuccelli, R., Caulmare, J., & Greenhalgh, P. (2019). Continuous in-line virus inactivation for next generation bioprocessing. *Biotechnology Journal*, *14*(2), e1700718. https://doi.org/10.1002/biot.201700718

Gjoka, X., Gantier, R., & Schofield, M. (2017). Transfer of a three-step mAb chromatography process from batch to continuous: Optimizing productivity to minimize consumable requirements. *Journal of Biotechnology*, *242*, 11–18. https://doi.org/10.1016/j.jbiotec.2016.12.005

Gjoka, X., Rogler, K., Martino, R. A., Gantier, R., & Schofield, M. (2015). A straightforward methodology for designing continuous monoclonal antibody capture multi-column chromatography processes. *Journal of Chromatography A*, *1416*, 38–46.

Glassey, J., Gernaey, K. V., Clemens, C., Schulz, T. W., Oliveira, R., Striedner, G., & Mandenius, C. F. (2011). Process analytical technology (PAT) for biopharmaceuticals. *Biotechnology Journal*, *6*(4), 369–377. https://doi.org/10.1002/biot.201000356

Godawat, R., Konstantinov, K., Rohani, M., & Warikoo, V. (2015). End-to-end integrated fully continuous production of recombinant monoclonal antibodies. *Journal of Biotechnology*, *213*, 13–19. https://doi.org/10.1016/j.jbiotec.2015.06.393

Gomis-Fons, J., Lofgren, A., Andersson, N., Nilsson, B., Berghard, L., & Wood, S. (2019). Integration of a complete downstream process for the automated lab-scale production of a recombinant protein. *Journal of Biotechnology*, *301*, 45–51. https://doi.org/10.1016/j.jbiotec.2019.05.013

Granicher, G., Coronel, J., Trampler, F., Jordan, I., Genzel, Y., & Reichl, U. (2020). Performance of an acoustic settler versus a hollow fiber-based ATF technology for influenza virus production in perfusion.

Applied Microbiology and Biotechnology, *104*(11), 4877–4888. https://doi.org/10.1007/s00253-020-10596-x

Gronemeyer, P., Thiess, H., Zobel-Roos, S., Ditz, R., & Strube, J (2017). Integration of Upstream and Downstream in Continuous Biomanufacturing, *Continuous Biomanufacturing-Innovative Technologies and Methods* (pp. 481–510). John Wiley & Sons, Ltd.

Gryniewicz, C. M., & Kauffman, J. F. (2008). Multivariate calibration of covalent aggregate fraction to the Raman spectrum of regular human insulin. *Journal of Pharmaceutical Sciences*, *97*(9), 3727–3734. https://doi.org/10.1002/jps.21326

Hebbi, V., Roy, S., Rathore, A. S., & Shukla, A. (2020). Modeling and prediction of excipient and pH drifts during ultrafiltration/diafiltration of monoclonal antibody biotherapeutic for high concentration formulations. *Separation and Purification Technology*, *238*, 116392.

Hogwood, C. E., Tait, A. S., Koloteva-Levine, N., Bracewell, D. G., & Smales, C. M. (2013). The dynamics of the CHO host cell protein profile during clarification and protein A capture in a platform antibody purification process. *Biotechnology and Bioengineering*, *110*(1), 240–251. https://doi.org/10.1002/bit.24607

Hummel, J., Pagkaliwangan, M., Gjoka, X., Davidovits, T., Stock, R., Ransohoff, T., Gantier, R., & Schofield, M. J. (2019). Modeling the downstream processing of monoclonal antibodies reveals cost advantages for continuous methods for a broad range of manufacturing scale. *Biotechnology Journal*, *14*(2):e1700665.

Kamga, M. H., Cattaneo, M., & Yoon, S. (2018). Integrated continuous biomanufacturing platform with ATF perfusion and one column chromatography operation for optimum resin utilization and productivity. *Preparative Biochemistry and Biotechnology*, *48*(5), 383–390. https://doi.org/10.1080/10826068.2018.1446151

Kamga, M. H., Woo Lee, H., Liu, J., & Yoon, S. (2013). Quantification of protein mixture in chromatographic separation using multi-wavelength UV spectra. *Biotechnology Progress*, *29*(3), 664–671. https://doi.org/10.1002/btpr.1712

Karst, D. J., Steinebach, F., Soos, M., & Morbidelli, M. (2017). Process performance and product quality in an integrated continuous antibody production process. *Biotechnology and Bioengineering*, *114*(2), 298–307. https://doi.org/10.1002/bit.26069

Keller, W. R., Evans, S. T., Ferreira, G., Robbins, D., & Cramer, S. M. (2015). Use of MiniColumns for linear isotherm parameter estimation and prediction of benchtop column performance. *Journal of Chromatography A*, *1418*, 94–102.

Kiesewetter, A., Menstell, P., Peeck, L. H., & Stein, A. (2016). Development of pseudo-linear gradient elution for high-throughput resin selectivity screening in RoboColumn® Format. *Biotechnology Progress*, *32*(6), 1503–1519.

Konstantinov, K. B., & Cooney, C. L. (2015). White paper on continuous bioprocessing. May 20–21, 2014 Continuous Manufacturing Symposium. *Journal of Pharmaceutical Sciences*, *104*(3), 813–820. https://doi.org/10.1002/jps.24268

Kornecki, M., & Strube, J. (2018). Process analytical technology for advanced process control in biologics manufacturing with the aid of macroscopic kinetic modeling. *Bioengineering (Basel)*, *5*(1), 25. https://doi.org/10.3390/bioengineering5010025

Kumar, V., Leweke, S., von Lieres, E., & Rathore, A. S. (2015). Mechanistic modeling of ion-exchange process chromatography of charge variants of monoclonal antibody products. *Journal of Chromatography A*, *1426*, 140–153.

Kwon, T., Prentice, H., de Oliveira, J., Madziva, N., Warkiani, M. E., Hamel, J.-F. P., & Han, J. (2017). Microfluidic cell retention device for perfusion of mammalian suspension culture. *Scientific Reports*, *7*(1), 1–11.

Le, K., Tan, C., Le, H., Tat, J., Zasadzinska, E., Diep, J., Zastrow, R., Chen, C., & Stevens, J. (2020). Assuring clonality on the beacon digital cell line development platform. *Biotechnology Journal*, *15*(1), 1900247.

Li, L., & Ismagilov, R. F. (2010). Protein crystallization using microfluidic technologies based on valves, droplets, and SlipChip. *Annual Reviews of Biophysics*, *39*, 139–158.

Li, M. Y., Ebel, B., Paris, C., Chauchard, F., Guedon, E., & Marc, A. (2018). Real-time monitoring of antibody glycosylation site occupancy by in situ Raman spectroscopy during bioreactor CHO cell cultures. *Biotechnology Progress*, *34*(2), 486–493. https://doi.org/10.1002/btpr.2604

Li, S. W., Song, H. P., & Leng, Y. (2014). Rapid determination of lovastatin in the fermentation broth of Aspergillus terreus using dual-wavelength UV spectrophotometry. *Pharmacien Biologiste*, *52*(1), 129–135. https://doi.org/10.3109/13880209.2013.833947

Liu, Y., Fernandez, J., Pu, Z., Zhang, H., Cao, L., Aguilar, I., Ritz, D., Luo, R., Read, A., Laures, A., Lan, K., Ubiera, A., Smith, A., Patel, P., & Liu, A. (2020). Simultaneous monitoring and comparison of multiple product quality attributes for cell culture processes at different scales using a LC/MS/MS based multi-attribute method. *Journal of Pharmaceutical Sciences*, *109*(11), 3319–3329. https://doi.org/10.1016/j.xphs.2020.07.029

Lofgren, A. L., Fons, J. G., Andersson, N., Nilsson, B., Berghard, L., & Hagglund, C. L. H. (2020). An integrated continuous downstream process with real-time control: A case study with periodic countercurrent chromatography and continuous virus inactivation. https://doi.org/10.22541/au.160029924.40393430

Lute, S., Kozaili, J., Johnson, S., Kobayashi, K., & Strauss, D. (2020). Development of small-scale models to understand the impact of continuous downstream bioprocessing on integrated virus filtration. *Biotechnology Progress*, *36*(3), e2962. https://doi.org/10.1002/btpr.2962

Mandenius, C.-F., & Gustavsson, R. (2015). Mini-review: Soft sensors as means for PAT in the manufacture of bio-therapeutics. *Journal of Chemical Technology & Biotechnology*, *90*(2), 215–227. https://doi.org/10.1002/jctb.4477

Martins, D. L., Sencar, J., Hammerschmidt, N., Tille, B., Kinderman, J., Kreil, T. R., & Jungbauer, A. (2019). Continuous solvent/detergent virus inactivation using a packed-bed reactor. *Biotechnology Journal*, *14*(8), e1800646. https://doi.org/10.1002/biot.201800646

McDonald, P., Tran, B., Williams, C. R., Wong, M., Zhao, T., Kelley, B. D., & Lester, P. (2016). The rapid identification of elution conditions for therapeutic antibodies from cation-exchange chromatography resins using high-throughput screening. *Journal of Chromatography A*, *1433*, 66–74.

Mehling, M., & Tay, S. (2014). Microfluidic cell culture. *Current Opinion in Biotechnology*, *25*, 95–102.

Nagy, B., Farkas, A., Gyurkes, M., Komaromy-Hiller, S., Demuth, B., Szabo, B., Nusser, D., Borbas, E., Marosi, G., & Nagy, Z. K. (2017). In-line Raman spectroscopic monitoring and feedback control of a continuous twin-screw pharmaceutical powder blending and tableting process. *International Journal of Pharmaceutics*, *530*(1–2), 21–29. https://doi.org/10.1016/j.ijpharm.2017.07.041

Nfor, B. K., Ahamed, T., van Dedem, G. W., Verhaert, P. D., van der Wielen, L. A., Eppink, M. H., van de Sandt, E. J., & Ottens, M. (2013). Model-based rational methodology for protein purification process synthesis. *Chemical Engineering Science*, *89*, 185–195.

Nfor, B. K., Noverraz, M., Chilamkurthi, S., Verhaert, P. D., van der Wielen, L. A., & Ottens, M. (2010). High-throughput isotherm determination and thermodynamic modeling of protein adsorption on mixed mode adsorbents. *Journal of Chromatography A*, *1217*(44), 6829–6850. https://doi.org/10.1016/j.chroma.2010.07.069

Nfor, B. K., Ripić, J., van der Padt, A., Jacobs, M., & Ottens, M. (2012). Model-based high-throughput process development for chromatographic whey proteins separation. *Biotechnology Journal*, *7*(10), 1221–1232.

Oliveira, A. L. (2019). Biotechnology, big data and artificial intelligence. *Biotechnology Journal*, *14*(8), 1800613.

Orozco, R., Godfrey, S., Coffman, J., Amarikwa, L., Parker, S., Hernandez, L., Wachuku, C., Mai, B., Song, B., Hoskatti, S., Asong, J., Shamlou, P., Bardliving, C., & Fiadeiro, M. (2017). Design, construction, and optimization of a novel, modular, and scalable incubation chamber for continuous viral inactivation. *Biotechnology Progress*, *33*(4), 954–965. https://doi.org/10.1002/btpr.2442

Parker, S. A., Amarikwa, L., Vehar, K., Orozco, R., Godfrey, S., Coffman, J., Shamlou, P., & Bardliving, C. L. (2018). Design of a novel continuous flow reactor for low pH viral inactivation. *Biotechnology and Bioengineering*, *115*(3), 606–616. https://doi.org/10.1002/bit.26497

Patel, B. A., Gospodarek, A., Larkin, M., Kenrick, S. A., Haverick, M. A., Tugcu, N., Brower, M. A., & Richardson, D. D. (2018). Multi-angle light scattering as a process analytical technology measuring real-time molecular weight for downstream process control. *mAbs*, *10*(7), 945–950. https://doi.org/10.1080/19420862.2018.1505178

Pinto, I. F., Santos, D., Soares, R., Aires-Barros, M., Chu, V., Azevedo, A., & Conde, J. (2018). A regenerable microfluidic device with integrated valves and thin-film photodiodes for rapid optimization of chromatography conditions. *Sensors and Actuators B: Chemical*, *255*, 3636–3646.

Pinto, N. D. S., Napoli, W. N., & Brower, M. (2020). Impact of micro and macroporous TFF membranes on product sieving and chromatography loading for perfusion cell culture. *Biotechnology and Bioengineering*, *117*(1), 117–124. https://doi.org/10.1002/bit.27192

Pirrung, S. M., Parruca da Cruz, D., Hanke, A. T., Berends, C., van Beckhoven, R. F., Eppink, M. H., & Ottens, M. (2018). Chromatographic parameter determination for complex biological feedstocks. *Biotechnology Progress*, *34*(4), 1006–1018.

Pirrung, S. M., van der Wielen, L. A., van Beckhoven, R. F., van de Sandt, E. J., Eppink, M. H., & Ottens, M. (2017). Optimization of biopharmaceutical downstream processes supported by mechanistic models and artificial neural networks. *Biotechnology Progress*, *33*(3), 696–707.

Rathore, A. S. (2009). Roadmap for implementation of quality by design (QbD) for biotechnology products. *Trends in Biotechnology*, *27*(9), 546–553.

Rathore, A. S., Agarwal, H., Sharma, A. K., Pathak, M., & Muthukumar, S. (2015). Continuous processing for production of biopharmaceuticals. *Preparative Biochemistry and Biotechnology*, *45*(8), 836–849. https://doi.org/10.1080/10826068.2014.985834

Rathore, A. S., Wood, R., Sharma, A., & Dermawan, S. (2008). Case study and application of process analytical technology (PAT) towards bioprocessing: II. Use of ultra-performance liquid chromatography (UPLC) for making real-time pooling decisions for process chromatography. *Biotechnology and Bioengineering*, *101*(6), 1366–1374. https://doi.org/10.1002/bit.21982

Rathore, A. S., Yu, M., Yeboah, S., & Sharma, A. (2008). Case study and application of process analytical technology (PAT) towards bioprocessing: use of on-line high-performance liquid chromatography (HPLC) for making real-time pooling decisions for process chromatography. *Biotechnology and Bioengineering*, *100*(2), 306–316. https://doi.org/10.1002/bit.21759

Read, E. K., Park, J. T., Shah, R. B., Riley, B. S., Brorson, K. A., & Rathore, A. S. (2010). Process analytical technology (PAT) for biopharmaceutical products: Part I. Concepts and applications. *Biotechnology and Bioengineering*, *105*(2), 276–284. https://doi.org/10.1002/bit.22528

Rho, H. S., Hanke, A. T., Ottens, M., & Gardeniers, H. (2017). A microfluidic device for the batch adsorption of a protein on adsorbent particles. *Analyst*, *142*(19), 3656–3665. https://doi.org/10.1039/c7an00917h

Sandner, V., Pybus, L. P., McCreath, G., & Glassey, J. (2019). Scale-down model development in Ambr systems: An industrial perspective. *Biotechnology Journal*, *14*(4), 1700766.

Sauer, D. G., Melcher, M., Mosor, M., Walch, N., Berkemeyer, M., Scharl-Hirsch, T., Leisch, F., Jungbauer, A., & Durauer, A. (2019). Real-time monitoring and model-based prediction of purity and quantity during a chromatographic capture of fibroblast growth factor 2. *Biotechnology and Bioengineering*, *116*(8), 1999–2009. https://doi.org/10.1002/bit.26984

Schmidt, P. M., Abdo, M., Butcher, R. E., Yap, M.-Y., Scotney, P. D., Ramunno, M. L., Martin-Roussety, G., Owczarek, C., Hardy, M. P., & Chen, C.-G. (2016). A robust robotic high-throughput antibody purification platform. *Journal of Chromatography A*, *1455*, 9–19.

Schwarz, H., Zhang, Y., Zhan, C., Malm, M., Field, R., Turner, R., Sellick, C., Varley, P., Rockberg, J., & Chotteau, V. (2020). Small-scale bioreactor supports high-density HEK293 cell perfusion culture for the production of recombinant erythropoietin. *Journal of Biotechnology*, *309*, 44–52.

Sharma, A., Chilin, D., & Rathore, A. S. (2006). Applying process analytical technology to biotech unit operations. *BioPharm International*, *19*(8), 48–57.

Shi, S., Condon, R. G., Deng, L., Saunders, J., Hung, F., Tsao, Y.-S., & Liu, Z. (2011). A high-throughput automated platform for the development of manufacturing cell lines for protein therapeutics. *Journal of Visualized Experiments*, *55*, e3010.

Shukla, A. A., Wolfe, L. S., Mostafa, S. S., & Norman, C. (2017). Evolving trends in mAb production processes. *Bioengineering & Translational Medicine*, *2*(1), 58–69. https://doi.org/10.1002/btm2.10061

Silva, D., Azevedo, A., Fernandes, P., Chu, V., Conde, J., & Aires-Barros, M. (2014). Determination of aqueous two phase system binodal curves using a microfluidic device. *Journal of Chromatography A*, *1370*, 115–120.

Sisodiya, V. N., Lequieu, J., Rodriguez, M., McDonald, P., & Lazzareschi, K. P. (2012). Studying host cell protein interactions with monoclonal antibodies using high throughput protein A chromatography. *Biotechnology Journal*, *7*(10), 1233–1241.

Somasundaram, B., Pleitt, K., Shave, E., Baker, K., & Lua, L. H. L. (2018). Progression of continuous downstream processing of monoclonal antibodies: Current trends and challenges. *Biotechnology and Bioengineering*, *115*(12), 2893–2907. https://doi.org/10.1002/bit.26812

Steinhoff, R. F., Karst, D. J., Steinebach, F., Kopp, M. R., Schmidt, G. W., Stettler, A., Krismer, J., Soos, M., Pabst, M., Hierlemann, A., Morbidelli, M., & Zenobi, R. (2016). Microarray-based MALDI-TOF mass spectrometry enables monitoring of monoclonal antibody production in batch and perfusion cell cultures. *Methods*, *104*, 33–40. https://doi.org/10.1016/j.ymeth.2015.12.011

Strnad, J., Brinc, M., Spudić, V., Jelnikar, N., Mirnik, L., Čarman, B., & Kravanja, Z. (2010). Optimization of cultivation conditions in spin tubes for Chinese hamster ovary cells producing erythropoietin and the comparison of glycosylation patterns in different cultivation vessels. *Biotechnology Progress*, *26*(3), 653–663.

Tait, A. S., Aucamp, J. P., Bugeon, A., & Hoare, M. (2009). Ultra scale-down prediction using microwell technology of the industrial scale clarification characteristics by centrifugation of mammalian cell broths. *Biotechnology and Bioengineering*, *104*(2), 321–331. https://doi.org/10.1002/bit.22393

Tang, A., Ramos, I., Newell, K., & Stewart, K. D. (2020). A novel high-throughput process development screening tool for virus filtration. *Journal of Membrane Science*, *611*, 611. https://doi.org/10.1016/j.memsci.2020.118330

Thakur, G., Hebbi, V., & Rathore, A. S. (2020). An NIR-based PAT approach for real-time control of loading in protein A chromatography in continuous manufacturing of monoclonal antibodies. *Biotechnology and Bioengineering*, *117*(3), 673–686. https://doi.org/10.1002/bit.27236

Thakur, G., Thori, S., & Rathore, A. S. (2020). Implementing PAT for single-pass tangential flow ultrafiltration for continuous manufacturing of monoclonal antibodies. *Journal of Membrane Science*, *613*, 118492. https://doi.org/10.1016/j.memsci.2020.118492

Treier, K., Hansen, S., Richter, C., Diederich, P., Hubbuch, J., & Lester, P. (2012). High-throughput methods for miniaturization and automation of monoclonal antibody purification processes. *Biotechnology Progress*, *28*(3), 723–732. https://doi.org/10.1002/btpr.1533

U.S. Department of Health and Human Services, F. a. D. A. F., Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), Office of Regulatory Affairs (ORA). (2004). PAT—A framework for innovative pharmaceutical development manufacturing and quality assurance.

U.S. Department of Health and Human Services, F. a. D. A. F., Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), Office of Regulatory Affairs (ORA). (2011). Guidance for industry: Process validation: General principles and practices.

Walther, J., Godawat, R., Hwang, C., Abe, Y., Sinclair, A., & Konstantinov, K. (2015). The business impact of an integrated continuous biomanufacturing platform for recombinant protein production. *Journal of Biotechnology*, *213*, 3–12. https://doi.org/10.1016/j.jbiotec.2015.05.010

Wang, G., Briskot, T., Hahn, T., Baumann, P., & Hubbuch, J. (2017). Estimation of adsorption isotherm and mass transfer parameters in protein chromatography using artificial neural networks. *Journal of Chromatography A*, *1487*, 211–217.

Wasalathanthri, D. P., Rehmann, M. S., Song, Y., Gu, Y., Mi, L., Shao, C., Chemmalil, L., Lee, J., Ghose, S., Borys, M. C., Ding, J., & Li, Z. J. (2020). Technology outlook for real-time quality attribute and process parameter monitoring in biopharmaceutical development—A review. *Biotechnology and Bioengineering*, *117*(10), 3182–3198. https://doi.org/10.1002/bit.27461

Wiendahl, M., Schulze Wierling, P., Nielsen, J., Fomsgaard Christensen, D., Krarup, J., Staby, A., & Hubbuch, J. (2008). High throughput screening for the design and optimization of chromatographic processes—Miniaturization, automation and parallelization of breakthrough and elution studies. *Chemical Engineering & Technology*, *31*(6), 893–903. https://doi.org/10.1002/ceat.200800167

Xu, P., Clark, C., Ryder, T., Sparks, C., Zhou, J., Wang, M., Russell, R., & Scott, C. (2017). Characterization of TAP Ambr 250 disposable bioreactors, as a reliable scale-down model for biologics process development. *Biotechnology Progress*, *33*(2), 478–489.

Yahia, B. B., Malphettes, L., & Heinzle, E. (2015). Macroscopic modeling of mammalian cell growth and metabolism. *Applied Microbiology and Biotechnology*, *99*(17), 7009–7024.

Zelger, M., Pan, S., Jungbauer, A., & Hahn, R. (2016). Real-time monitoring of protein precipitation in a tubular reactor for continuous bioprocessing. *Process Biochemistry*, *51*(10), 1610–1621. https://doi.org/10.1016/j.procbio.2016.06.018

Zhu, Y., Chen, Q., Shao, L., Jia, Y., & Zhang, X. (2020). Microfluidic immobilized enzyme reactors for continuous biocatalysis. *Reaction Chemistry & Engineering*, *5*(1), 9–32.

Zydney, A. L. (2016). Continuous downstream processing for high-value biological products: A review. *Biotechnology and Bioengineering*, *113*(3), 465–475. https://doi.org/10.1002/bit.25695

How to cite this article: São Pedro, M. N., Silva, T. C., Patil, R., & Ottens, M. (2021). White paper on high-throughput process development for integrated continuous biomanufacturing. *Biotechnology Bioengineering*, 118, 3275–3286. https://doi.org/10.1002/bit.27757