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RESEARCH ARTICLE

Bioseparations and Downstream Processing

Evaluating end-to-end continuous antibody manufacture with column-free capture alternatives from economic, environmental, and robustness perspectives

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Abstract

Process intensification efforts have renewed interest in the potential of end-to-end continuous manufacture with column-free capture alternatives. This article describes a decisional tool that encompasses mass balance and design equations, process economics, stochastic simulation and multi-criteria decision-making and enables the evaluation of different batch, and continuous flowsheets for monoclonal antibody (mAb) manufacture. The traditional batch process was compared with end-to-end continuous bioprocesses with either protein A capture or column-free capture employing aqueous two-phase extraction or precipitation from economic, environmental, and robustness perspectives. The cost of goods analysis predicted that continuous flowsheets could offer substantial cost savings (~20%–40%) over the batch process at low and medium annual commercial demands (100–500 kg); however, at tonnage demands they resulted in either comparable or higher costs. Comparing the continuous options, the continuous flowsheets with protein A or precipitation yielded similar COG/g values, while aqueous two-phase extraction presented higher costs. The analysis of overall process mass intensities accounting for water and consumables suggested that the continuous flowsheet with protein A would result in the lowest environmental burden. When the economic, environmental, and operational criteria were reconciled using multi-criteria decision-making analysis, the continuous protein A-based flowsheet was found to be the most favorable. A target analysis highlighted the need for process improvements in the following parameters to reduce the manufacturing costs of the continuous column-free capture options below that of protein A: the perfusion volumetric productivity, the harvested cell culture fluid percentage in column-free operations, the column-free step yields along with the implementation of buffer concentrates.

KEYWORDS

aqueous two-phase extraction, cost of goods, monoclonal antibody manufacturing, precipitation, process economics, protein A chromatography

1 | INTRODUCTION

The biopharmaceutical sector has demonstrated sustained progress in addressing the gaps that would enable a shift from batch to integrated continuous bioprocesses, driven by the desire to increase productivity and flexibility while reducing costs (e.g., Refs. 1–4). Net zero ambitions are also encouraging the sector to consider more sustainable and greener processes,[5] and continuous manufacturing has the potential to act as an enabler for smaller facility footprints that may facilitate achieving these targets. Examples include recent investments in continuous processing plants for biologics production by both biopharma companies (e.g., Sanofi, Framingham, MA) and contract development and manufacturing organizations (e.g., Fujifilm Diosynth Biotechnologies, Billingham, UK).[6,7] As monoclonal antibodies (mAbs) represent the fastest growing biopharmaceutical market segment, these have also been the prime testbed for continuous manufacturing. Process intensification efforts have renewed interest in using continuous column-free alternatives for mAb capture with the aim of eliminating the high upfront costs associated with protein A (ProA) resins. However, while technical evaluations of continuous column-free processes in mAb production have been widely reported (e.g., Refs. 8–11), the assessment of economic, environmental, and robustness perspectives to support decision-making is limited. This article describes the investigation of several mAb production flowsheets using a decisional tool that encompasses not only cost of goods (COG) analysis but also the calculation of environmental, operational, and robustness metrics for batch and continuous flowsheets, allowing for a more comprehensive analysis of the trade-offs associated with both column-based and column-free options.

A number of studies have explored the economics of batch versus continuous processes for mAbs with the standard ProA capture. These include decisional tools studies using bespoke algorithms and software (e.g., Refs. 2,12–14) and studies with off-the-shelf software (e.g., Refs. 15–17). These articles have highlighted fixed capital investment (FCI) and COG savings with continuous processes, with the latter ranging between 10% and 65%, and addressed the nuances between hybrid and end-to-end continuous flowsheets at different production scales.

Interest in column-free alternatives to ProA chromatography for mAb capture has been cyclical over the years with recent focus on their potential when operated in continuous mode. In spite of the renewed attention given to continuous column-free options, such as membrane chromatography, flocculation, precipitation, and aqueous two-phase extraction (ATPE), there is a lack of a clear business case for the implementation of such techniques in large scale. This is further complicated by the relatively low technology readiness and maturity levels of continuous manufacturing, and especially continuous downstream processing. The decisional tool described in this article was designed to assess the implementation of ATPE and precipitation (PP) and to help determine how these can compete with standard ProA chromatography in end-to-end continuous mAb production.

The growing interest in ATPE in mAb processing is linked to its high biocompatibility with biomolecules (phases are 80%–90% water), high capacity and ease of scaling-up.[18,19] Recovery yields higher than 80% and purities higher than 97% have been reported in continuous mode.[20] Also, continuous precipitation in a coiled flow inverter reactor was presented as a viable mAb capture technique, with a recovery yield higher than 95% for the step in batch mode.[10,21] In continuous mode, the trend has been to use this technique to precipitate the product (instead of impurities) and the step recovery yields and purity are also above 95%.[8,22] Cost studies have evaluated hybrid batch/continuous processes with either continuous ATPE[20] or continuous precipitation[23] in mAb bioprocessing. However, further research regarding the potential of column-free technologies integrated in a fully end-to-end continuous process with GMP equipment is needed, as well as a fair comparison with the current state-of-the-art of continuous ProA chromatography-based capture. Moreover, companies need more systematic methods to assist with decision-making that embrace several perspectives as they endeavor to balance enhanced technical performance with the resultant impact on costs, environmental metrics, and operational ease of implementation.

This article presents the application of a decisional tool designed to execute detailed mass balances and facility sizing based on relevant academic and industrial technical data and assumptions for continuous manufacturing with column-based and column-free mAb capture steps and combines a multitude of evaluation perspectives. The tool was applied to a set of industrially-relevant case studies that address the following topical questions: *"How does cost of goods compare for batch and continuous mAb flowsheets?"*, *"Are there environmental benefits moving from batch to continuous processing and column-based to column-free mAb capture?"*, *"How does batch-to-batch variability influence the robustness of different flowsheets?"*, and finally *"What process improvements are required to decrease the costs of column-free alternatives?"*. This study and the predictive modeling results bring transparency to the assessment of the potential of newer technologies integrated in a fully continuous mAb production process.

2 | MATERIALS AND METHODS

2.1 | Decisional tool description

A decisional tool was built in Python to model the economic, environmental, and operational features associated with different mAb production flowsheets. The tool integrated algorithms for bioprocess economics, environmental analysis, uncertainty analysis via Monte Carlo simulation and multi-criteria decision-making. The tool structure, database, and process economic equations built on previous UCL work.[2,12,24,25] The tool to model end-to-end continuous bioprocesses presented by Mahal et al.[2] was extended to integrate the following new features: (i) mass balance and design equations for aqueous-two phase extraction and precipitation; (ii) equipment costs and default process parameter values for column-free capture operations and; (iii) calculations for environmental metrics with an updated database including masses of consumables. Table 1 depicts the key economic and environmental equations featured in the tool as well as the key process parameters. The FCI was computed using the Lang factor and the total equipment purchase cost. The COG comprised

TABLE 1 Key design equations and process-specific input assumptions in the COG model for Batch-ProA, Conti-ProA, Conti-ATPE, and Conti-PP flowsheets.

Key design equations	
Chromatography column volume, CV (L)	$\frac{m_{\text{out perfusion}}}{\text{DBC}\times N_{\text{col}}\times N_{\text{cycles}}}$
Diameter of chromatography column, D_{column} (cm)	$\sqrt{\frac{\text{CV}\times 4000}{\pi\times BH}}$
Flowrate of PEG, salts, and crude feedstock loaded to the ATPE extractor or PP static mixer, $Q_{\text{in ATPE/PP}}$ (L/h)	$\frac{Q_{\text{out perfusion}}}{\text{HCCF}\%}$
Total volume of ATPE/PP system, $V_{\text{ATPE/PP system}}$ (L)	$\frac{V_{\text{out perfusion}}}{\text{HCCF}\%}$
Diameter of ATPE extractor or PP static mixer, $D_{\text{extractor/static mixer}}$ (cm)	$\sqrt{\frac{Q_{\text{in total}}\times 4000\times t_{\text{res}}}{\pi\times L}}$
Volume out of the ATPE extractor, $V_{\text{out ATPE}}$ (L)	$\frac{V_{\text{ATPE system}}}{\text{Ratio}_{\text{top/bottom}}}$

Process-specific data			
Unit operation	**Parameter**	**Batch**	**Continuous**
Cell culture	Culture Duration (days)	14	28
	Perfusion rate (vv/day)	-	1.5
	Volumetric productivity ($g/L_{vv}/d$)	0.4	3
	Max bioreactor volume (L)	20,000	2000
	Collected titer ($g/L_{harvest}$)	5	2
	Batches per year, $N_{batches}$	20	10
Protein A chrom.	Bed height, BH (cm)	20	10
	Loading capacity, DBC (g/L_{resin})	40	65
	Linear velocity (cm/h)	350	180
	Number of columns, N_{col}	1	3
	Resin reuse limit, N_{reuse} (cycles)	200	200
ATPE	HCCF (%)	-	18
	PEG (%)	-	9.6
	Phosphate (%)	-	13
	NaCl (%)	-	10
	$\text{Ratio}_{\text{top/bottom}}$	-	0.4
Precipitation	HCCF (%)	-	50
	PEG (%)	-	7
	$ZnCl_2$ (%)	-	10
VI	Concentration into VI (g/L)	~18	31.5
Final UFDF	Final target concentration (g/L)	30	30

Note: Collected titer is measured in grams of product per liter of harvested cell culture fluid. Volumetric productivity is measured in grams of product produced per liter of the bioreactor working volume per day. Perfusion rate is measured as the equivalent number of bioreactor vessel working volumes (vv) exchanged per day. $m_{\text{out bioreactor}}$: Mass of product out of the bioreactor per batch (Kg). $Q_{\text{out perfusion}}$: Flowrate of harvested cell culture fluid out from perfusion (L/h). HCCF%: Harvested cell culture fluid percentage in the ATPE/PP system. t_{res}: Residence time (h). $\text{Ratio}_{\text{top/bottom}}$: Volume ratio between top and bottom phases in ATPE system.

direct (reagents, consumables, labor, and quality control materials) and indirect (depreciation and facility-dependent overheads) costs. The tool determined the process mass intensity (PMI) indicators as environmental metrics; PMI was defined as the total mass of waste generated per total mass of product, categorized into water waste and consumables waste. Water waste was categorized further into process water (e.g., cell culture media and process buffers) and non-process water (e.g., CIP and SIP). The tool used a multi-criteria decision-making technique to combine the tool's financial and environmental metrics with operational metrics, such as ease of scale-up.

2.1.1 | Sensitivity and uncertainty analysis methodology

One-way sensitivity analyses were used to identify the key COG drivers. These were then used in the Monte Carlo simulations. The Monte Carlo simulation algorithm was coded in Python to enable distributions (e.g., triangular) to be applied to the designated input parameters and used a random number generator to create the set of iterations. The algorithm computed the likelihood of the COG output falling below different thresholds. A two-tailed *t*-test was performed to evaluate whether there was a significant difference between the COG/g distributions of the flowsheets, as indicated by the *p*-values and a chosen significance level of 0.05. The algorithm was used to perform 100 iterations per run, which was found to be sufficient to reach convergence. The number of iterations needed to reach convergence was determined by calculating the mean and standard deviation after each run (from $n = 2$) and monitoring when these values were within a tolerance of 5% from the global mean and standard deviation. The global mean and standard deviation corresponded to the values calculated after 1000 runs.

2.1.2 | Multi-criteria decision-making methodology

The multi-criteria decision-making (MCDM) technique incorporated in the decisional tool was based on the weighted sum method and was designed to provide an overall measure of attractiveness for each flowsheet that reconciled economic, environmental, and operational criteria.[12,14] The economic (COG and FCI) and environmental (water and consumables PMIs) ratings (x_{ij}) were directly obtained from the process economics model. The operational criteria identified for the analysis were robustness, ease of validation, ease of installation, ease of scale-up, and ease of operation. The relative rating values of each flowsheet at each operational criteria (x_{ij}) and the rank of importance of each criterion among all operational criteria (E_i) were gathered from a survey questionnaire sent to industry and academic experts on the field. The criteria expressing economic and environmental feasibilities were ranked equally within each criteria category. All rating values were standardized to a common dimensionless scale (r_{ij}) between 0 and 100 according to Equation (1).

$$r_{ij} = \frac{x_{ij} - x_{\text{iWorst}}}{x_{\text{iBest}} - x_{\text{iWorst}}} \times 100 \quad (1)$$

The weight of each criterion, E_i, was based on the rank of importance (most important weighs the most) and was then normalized as w_i according to Equation (2).

$$w_i = \frac{E_i}{\sum_{i=1} E_i}, \text{where } E_i = a, a-1, \ldots, 1 \text{ for rank} = 1, 2, \ldots, a. \quad (2)$$

The weighted score of each flowsheet for each criterion, y_{jk}, (i.e., economic, operational, and environmental) was derived using Equation (3).

$$y_{jk} = \sum_{i=1}^{n} r_{ij} \times w_i. \quad (3)$$

The overall aggregated score, S_j, was computed according to Equation (4). The ratios of importance of each criteria category (R_k) enabled the priorities of the economic, environmental, and operational criteria to be altered based on user preferences, where the sum of the R_k values was equal to 1 ($R_{\text{eco}} + R_{\text{env}} + R_{\text{op}} = 1$).

$$S_j = \sum_{k=1}^{n} y_{jk} \times R_k = y_{j,\text{eco}} \times R_{\text{eco}} + y_{j,\text{env}} \times R_{\text{env}} + y_{j,\text{op}} \times R_{\text{op}}, \quad (4)$$

where j: alternative (Conti-ProA, Conti-ATPE, and Conti-PP); k: criteria category (economic, environmental, and operational); i: criterion (COG, FCI, PMI, robustness, ease of validation, etc.); r_{ij}: standardized rating of alternative j in subcriterion i; x_{ij}: rating value of alternative j in subcriterion i; $x_{i\text{Worst/Best}}$: Worst/Best rating value for subcriteron i among all alternatives; w_i: normalized weight of criterion i in category k; E_i: weight of criterion i in category k (based on rank); a: number of rankings; y_{jk}: weighted score of each alternative j in category k; S_j: overall aggregated score of alternative j; and R_k: ratio of importance of criteria k.

2.2 | mAb production flowsheets

The tool was used to model and evaluate commercial mAb facilities using different production strategies across different annually required product demands. The case studies explored three antibody capture technologies: ProA affinity chromatography, ATPE, and product precipitation (PP). Figure 1 shows the flowsheets modeled in the decisional tool.

While Batch-ProA depicted a typical fed-batch process, the continuous flowsheets integrated a perfusion bioreactor that enabled the retention of the cells inside the bioreactor and, therefore, did not require centrifugation and depth filtration as primary recovery steps before mAb capture. The simulation of the batch and continuous flowsheets integrating ProA chromatography was described by Mahal et al.[2] In this article, in Conti-ATPE and Conti-PP, only the capture stage was re-designed, thus, the process modeling from the viral inactivation to the final inline diafiltration was kept as described by Mahal et al.[2]

For the capture stage, the multicolumn ProA chromatography modeled in Conti-ProA was replaced with multiple steps in Conti-ATPE and Conti-PP. In Conti-ATPE, the ATPE takes place in a glass column and the operation occurs countercurrently, where the top PEG-rich phase is continuously fed at the bottom of the column, while the bottom salt-rich phase is fed at the top of the column.[26] The product is recovered from the top PEG-rich phase. As the extraction led to a dilution of the stream, a concentration step (single-pass filtration) was included afterwards to concentrate the product phase. A subsequent diafiltration step was added for buffer exchange and removal of the PEG from the system before the viral inactivation.

In Conti-PP, the harvested cell culture fluid (HCCF) and zinc are continuously fed to a static mixer, where product precipitation occurs inline. A second static mixer was placed in series, to which PEG is added to promote the growth of precipitates.[22] The precipitates were then concentrated, washed and re-concentrated in a single continuous filtration unit to decrease the number of equipment required. After an in-line resolubilisation in another static mixer, a depth filtration was also included to avoid solid particles entering the remaining DSP. Before the viral inactivation, an extra concentration step was modeled to achieve the target concentration. All design equations are summarized in the Supporting Information.

All batch and continuous process trains were designed to produce 100–1000 kg per year. The cell culture size was calculated based on the annual demand required and on the overall yields computed for the DSP train in each flowsheet. Bioreactor sizes were adjusted based on vendor constraints with a maximum size of 20,000 L for stainless-steel bioreactors and 2000 L for single-use bioreactors. The single-use based continuous production flowsheets (Conti-ProA, Conti-ATPE, and Conti-PP) were compared with the reference batch stainless-steel facility type with ProA chromatography as capture step (Batch-ProA). For Batch-ProA, the equipment sizing was based on the mass entering each unit operation and all steps were carried out sequentially. For the continuous options, the sizing was based on the outlet flowrate of the previous unit operation in the process train and the end-to-end continuous process was achieved through keeping the product outlet and inlet flows between units constant to avoid surge vessels or hold-times. When sizing the continuous ATPE glass column, the height was kept constant, while for continuous precipitation the length of the static mixers[22] was kept constant. The sizing equations of the chromatography column, ATPE extractor and precipitation static mixers can be found in Table 1.

2.3 | Case study assumptions

2.3.1 | Process parameters

Table 1 summarizes the key input assumptions for each flowsheet. A 14-day fed-batch process with a 5 g/L titer was compared with a 28-day perfusion in the continuous mAb production strategies.

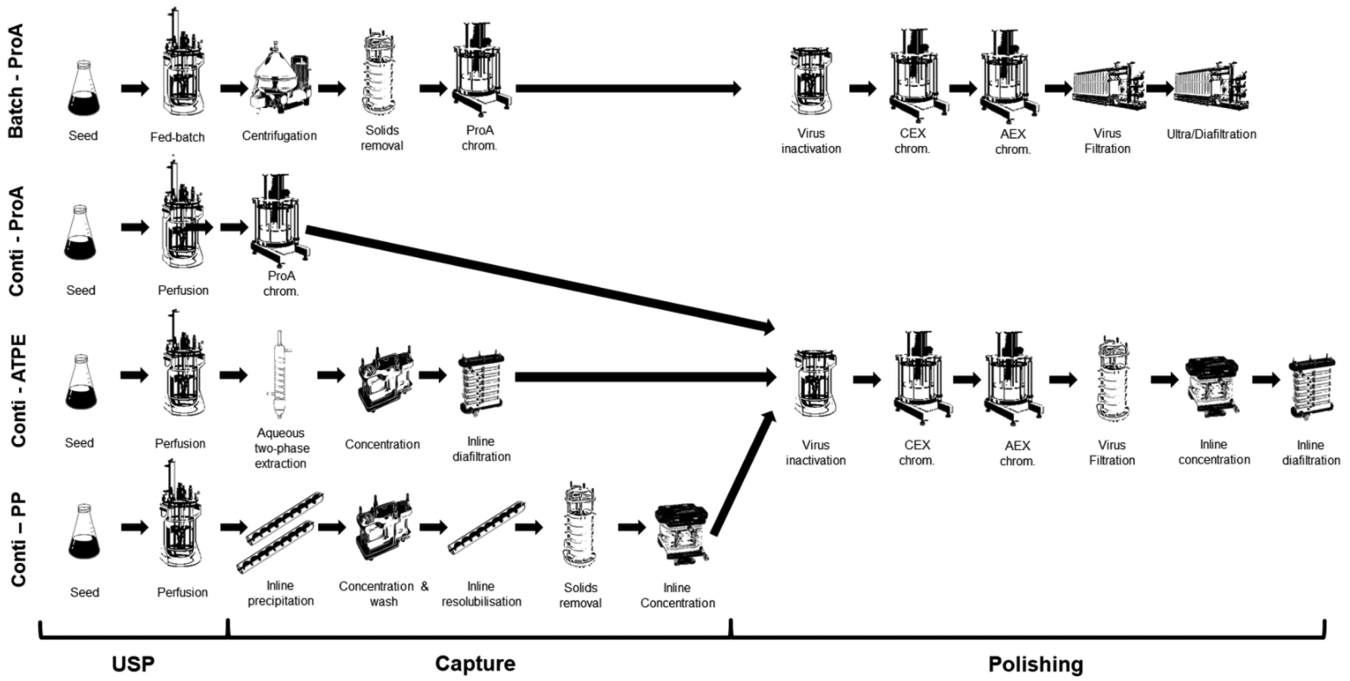


FIGURE 1 Process flowsheets studied in batch and continuous production of monoclonal antibodies. ProA: protein A; CEX: cation exchange; AEX: anion exchange; TFF: tangential flow filtration; SP-TFF: Single-pass tangential flow filtration. Batch-ProA: batch mAb production with ProA as capture step; Conti-ProA: continuous mAb production with ProA chromatography as capture step; Conti-ATPE: continuous mAb production with aqueous two-phase extraction as capture step; Conti-PP: continuous mAb production with product precipitation as capture step.

For continuous perfusion, the product collection starts only after the initial growth and ramp-up phase of 8 days and a 7-fold increase in volumetric productivity (3 g/L/d) was assumed over batch. For continuous multicolumn chromatography, higher resin loading capacities (ProA DBC = 65 g/L resin, AEX DBC = 100 g/L resin, and CEX DBC = 100 g/L resin) were assumed given the better resin capacity utilization compared with batch.[13,27] The binding capacities and prices for ProA resin in the different modes were selected to reflect the latest industry benchmarks.

For continuous ATPE and continuous precipitation, the percentage of HCCF (percentage of the final volume in the ATPE/PP system corresponding to the perfusion broth volume after adding the other components, such as PEG or salt) was assumed as 18%[26] and 50%,[22] respectively. The yield of continuous ProA chromatography in Conti-ProA was set as 95%, while the base case recovery of the ATPE step was 85%[26] and the wash yield in Conti-PP was 82%.[22] The resulting overall DSP yields were of 70%, 60%, and 55% for Conti-ProA, Conti-ATPE and Conti-PP, respectively. The product concentration prior to the viral inactivation step in Conti-ATPE and Conti-PP was set as the same found after ProA chromatography in Conti-ProA (32 g/L), so the size and time of the polishing stage would be kept constant across continuous flowsheets.

Some authors have shown comparable purity[26,28] when using alternatives to ProA chromatography in mAb capture, others state further work is required to improve impurity removal.[22] In this article, the economic potential of the flowsheets was explored on the basis that all flowsheets are able to meet the target purity specifications.

The batch and continuous labor requirements are as described by Mahal et al.[2] and three shifts per day are assumed with six operators per USP and per DSP shifts in Batch-ProA and three operators per USP and per DSP shifts in Conti-ProA, Conti-ATPE, and Conti-PP. In this article, the definition of "batch" in the continuous flowsheets is taken as the quantity of product delivered per cell culture run (10 batches per year). Similarly to what is described in Mahal et al.,[2] as a quality control batch release test in continuous is performed every 4 days on the material collected in that period of time, there are five "QC batches" per perfusion culture, which is taken into account when calculating the QC costs (35 k$ per batch release test).

In the present model, all process buffers were purchased for a fixed cost (no buffer preparation in-house). In single-use facilities, these buffers are stored in single-use bags (maximum capacity of 5000 L) and bag containers and trolleys are required to hold them in place where needed throughout the process train. These containers were considered in the indirect costs as part of the equipment purchase cost used in the calculation of the FCI.

2.3.2 | Uncertainty assumptions

Typical cell culture titer fluctuations are of ±20%.[12] This variation was also applied to the dynamic binding capacity (DBC) of ProA, as a way of simulating the influence that different required quantities of expensive resin could have on the COG. The specific column-free alternatives' parameters and ranges were discussed with experts in these technologies. Variations at the cell culture tire or volumetric productivity were translated into smaller/bigger/more/fewer bioreactors required and smaller/larger media consumption. The same effect was seen when considering uncertainty in the process step yields, as the

USP was redesigned to compensate for the product gain/loss during the downstream processing. The variation of the HCCF percentage in ATPE and PP impacted the dilution of the broth coming from perfusion, thus, the burden on the concentration steps required before the virus inactivation.

3 | RESULTS

The attractiveness of batch and continuous manufacturing strategies with different capture technologies was assessed using a decisional tool that captured the nuances of different modes of operation and different technology choices. The cost analysis was extended by evaluating each process's environmental burden and using stochastic uncertainty analysis to assess the robustness of the different scenarios under inherent process variability. An MCDM analysis was used to weigh up the financial, environmental, and operational attributes of each flowsheet. A final target analysis highlighted the process changes needed for alternative production strategies to become cost-competitive.

3.1 | Deterministic COG analysis of batch and continuous mAb processes with different capture technologies

The COG/g outputs from the deterministic analysis conducted with the decisional tool are shown in Figure 2a on a cost category basis for the batch and continuous mAb flowsheets. This figure shows that the continuous production flowsheets, whether ProA-based or column-free (Conti-ProA, Conti-ATPE, and Conti-PP) could offer COG savings of ~20%–40% compared with the standard batch flowsheet (Batch-ProA) at lower and medium scales (100 and 500 kg/year). In contrast, at higher scales (1000 kg/year) only Conti-ProA and Conti-PP presented a similar or slightly lower COG than Batch-ProA.

The cost savings with continuous flowsheets relative to batch at low and medium scales were driven by savings in indirect and reagent costs. The decrease in indirect costs can be attributed to the smaller equipment needed in continuous mode given the higher cell culture productivities; e.g., at 500 kg/year the indirect costs change from 56$/g for Batch-ProA to 25$/g for Conti-ProA, 31$/g for Conti-ATPE, and 24 $/g for Conti-PP. The savings in equipment costs switching from Batch-ProA to the continuous flowsheets could go up to 50% for 7-fold productivity differences. Also, as shown in Figure 2b, the savings associated with the absence of CIP cleaning procedures in SU facilities outweighed the 2 to 3-fold higher media consumption found for perfusion bioreactors and led to a 10%–60% cost reduction in reagents across scales and flowsheets (e.g., 500 kg/year reagents costs: 27$/g Batch-ProA, 21$/g Conti-ProA, 25$/g Conti-ATPE, and 26$/g Conti-PP). The savings in indirect and reagent costs were more significant than the increase in consumables costs (up to 2-fold) when using single-use continuous flowsheets (e.g., 500 kg/year consumables costs: 7$/g Batch-ProA; 11 $/g Conti-ProA; 13$/g Conti-ATPE, 10$/g Conti-PP).

Turning to the comparison at the higher 1000 kg/year demand, the lower COG savings with the continuous flowsheets were due to the need for multiple (two) parallel production trains. As the capacity of the SU bioreactor bags is limited (2000 L), when more than one bioreactor was required, an additional dedicated DSP train was simulated in parallel. At 1000 kg/year, Conti-ProA and Conti-PP presented COG savings of 8% and 3%, respectively, compared with Batch-ProA, which were not so significant given the typical accuracy of cost estimates. Due to the comparable indirect costs and higher consumables costs of Conti-ATPE compared with Batch-ProA at high scale, the ATPE-based flowsheet showed a COG/g increase of more than 10% compared with the batch case; this was the only scenario where a continuous flowsheet was found to perform worse than the batch flowsheet.

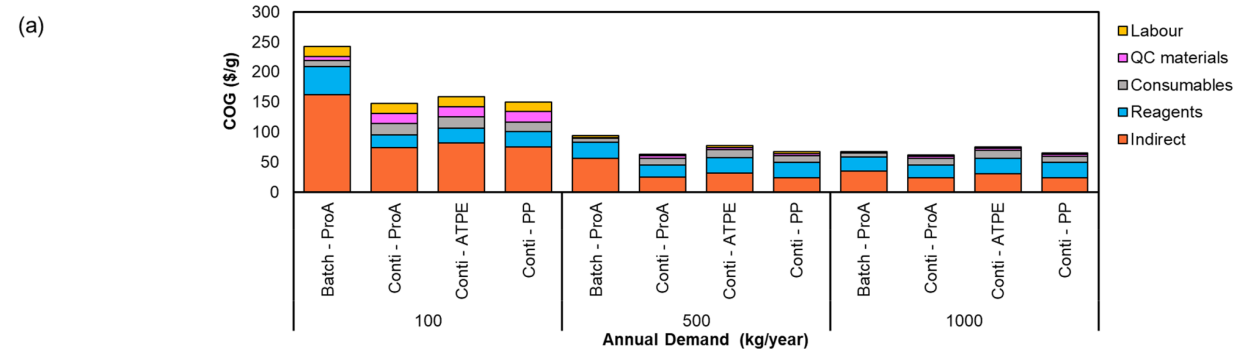
The cost comparison among continuous flowsheets in Figure 2a also showed that Conti-ProA was the strategy offering the lowest COG/g across scales, followed closely by Conti-PP (2%–6% higher COG). Conti-ATPE presented the highest COG/g (8%–22% higher COG than Conti-ProA) among all continuous flowsheets. For Conti ATPE, all cost categories were higher than Conti-ProA. This is mainly attributed to the HCCF dilution that drives up equipment costs (bag containers and filtration skids), consumables (SU bags), and reagents (ATPE-specific buffers and diafiltration buffers). For Conti PP, the overall cost was similar or slightly higher than Conti-ProA. While the reagents costs were 24% higher, driven by the larger media volumes (30% higher media costs than Conti-ProA), the consumables costs were lower (9%–14%), mainly due to the absence of ProA resin, and the total equipment purchase cost was similar.

Figure 3a depicts the COG breakdown by major stage (USP/capture/polishing). This highlights that the steps involved in the capture stage represented a significant proportion of the COG with a base value of ~30% for the conventional batch flowsheet. Moving from the conventional batch flowsheet (Batch-ProA) to continuous flowsheets with chromatography (Conti-ProA) or precipitation (Conti-PP) resulted in a reduction in the contribution of the capture stage to the overall COG from ~30% to <25%. In contrast, the continuous ATPE flowsheet (Conti-ATPE) resulted in a higher contribution of the capture stage to the overall COG than any of the other strategies (37%–44%).

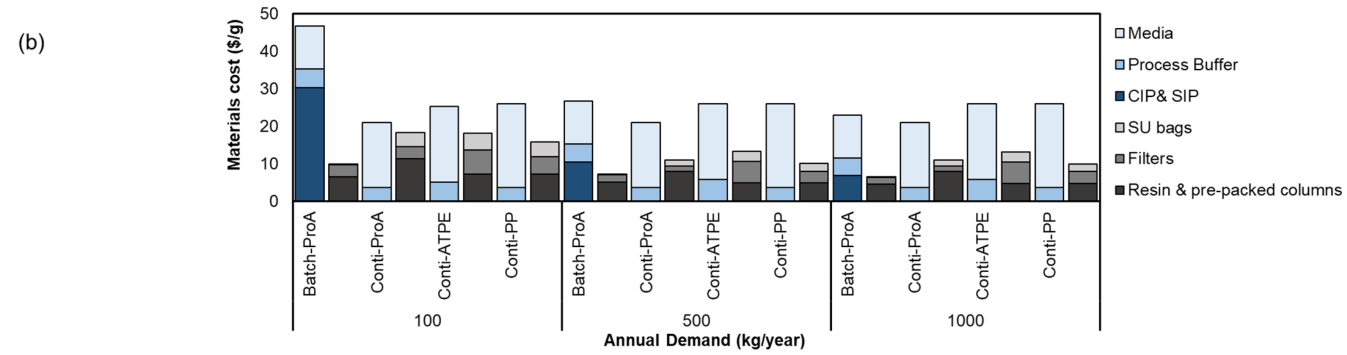
The cost category distributions by stage were investigated further, focusing on the stages most affected by the change in strategy—USP and capture. For the capture stage, both batch and continuous processes were dominated by the indirect costs that consumed over 50% of the capture COG; hence for capture stages, changes in capital equipment would have a larger impact than changes in materials, such as the resin. In contrast, for the USP stage, the move from batch to continuous flowsheets resulted in a shift in the USP COG distribution from being dominated by USP indirect costs (50%) in batch processes to USP materials (~70%–80%, predominantly culture media reagents) in continuous processes.

3.2 | Environmental analysis of batch and continuous mAb processes with different capture technologies

The potential environmental benefits moving from batch to continuous and from column-based to column-free mAb capture were



% difference in COG vs. Batch-ProA	–	–39%	–34%	–38%	–	–33%	–18%	–29%	–	–8%	+12%	–3%
% difference in COG vs. Conti-ProA	–	–	+8%	+2%	–	–	+22%	+6%	–	–	+22%	+6%
DSP yield	0.63	0.70	0.68	0.55	0.63	0.70	0.68	0.55	0.63	0.70	0.68	0.55
Reactor size (kL)	2	0.3	0.3	0.4	10	1.5	1.5	2	20	1.5	1.5	2
ProA chrom. scale (# columns, diameter in cm; CV in L)	1;45;32	3;13;1.2	–	–	1;80;100	3;25;5	–	–	1;110;190	3;25;5	–	–
Filtration (SPTFF) area (m^2)	–	–	3.5	1.5	–	–	37.5	7.5			37.5	7.5
Batches/campaign	20	10	10	10	20	10	10	10	20	20	20	20
Parallel trains	1	1	1	1	1	1	1	1	1	2	2	2
FCI ($M)	56	25	28	25	97	42	54	41	124	85	108	83
% indirect in total COG	67%	50%	51%	50%	59%	39%	40%	36%	53%	40%	41%	37%
% reagents in total COG	19%	14%	16%	17%	28%	33%	34%	39%	34%	34%	35%	40%
% consumables in total COG	4%	12%	12%	11%	8%	17%	17%	15%	9%	18%	18%	15%



% difference in reagents costs	vs. Batch-ProA	–	–55%	–44%	–44%		–22%	–3%	–3%		–8%	+13%	+13%
	vs. Conti-ProA	–	–	+24%	+24%		–	+24%	+24%		–	+24%	+24%
% difference in consumables costs	vs. Batch-ProA	–	+84%	+83%	+59%	–	+56%	+88%	+43%	–	+73%	+109%	+58%
	vs. Conti-ProA	–	–	+1%	–14%	–	–	+20%	–9%	–	–	+20%	–9%
% difference in equipment costs	vs. Batch-ProA	–	–55%	–41%	–54%	–	-55%	–15%	–53%	–	–30%	+33%	–27%
	vs. Conti-ProA	–	–	+33%	+4%	–	–	+90%	+4%	–	–	+90%	+4%

FIGURE 2 Breakdown of (a) COG/g on a cost category basis and (b) materials (reagents and consumables) cost for four mAb production flowsheets at 100, 500, and 1000 kg/year commercial scales. The fed-batch flowsheet is integrated in a stainless-steel based facility, while the continuous flowsheets are single-use based. The titer for fed-batch culture is 5 g/L and the perfusion volumetric productivities assumed in all continuous strategies was 3 g/L/day. The embedded table in (a) indicates the key parameters for each batch and continuous facility and the percentage of indirect, reagents, and consumables in each flowsheet's COG/g. The embedded table in (b) presents the percentage difference in equipment, reagents, and consumables costs between flowsheets.

evaluated by analyzing the environmental burden associated with each mAb flowsheet. The PMIs are shown in Figure 4 and are split into water and consumables PMI for the different production strategies. Continuous flowsheets offered a significant reduction in the overall PMI compared with the traditional batch process depending on scale, with Conti-ProA offering the most environmentally friendly strategy of all mAb production strategies. This was driven by the reduction in water PMI that outweighed any increases in consumables PMI since water PMI values were in the order of 1000's kg/kg while consumables PMI were significantly lower and in the order of 10's kg/kg.

Digging deeper into the analysis produces useful PMI benchmark values for the sector. Water PMIs for Batch-ProA were between 5000 and 17,000 kg/kg, while consumables PMIs ranged between 4 and 6 kg/kg, depending on the production scale. According to Figure 4a, the switch from batch to continuous flowsheets can lead to 2–8-fold lower water PMIs from high to low production scales (2200 kg/kg Conti-ProA, 3300 kg/kg Conti-ATPE, 3600 kg/kg Conti-PP across scales). As discussed in the cost analysis, the absence of CIP procedures in continuous single-use based facilities results in significant water savings compared with the batch stainless-steel based

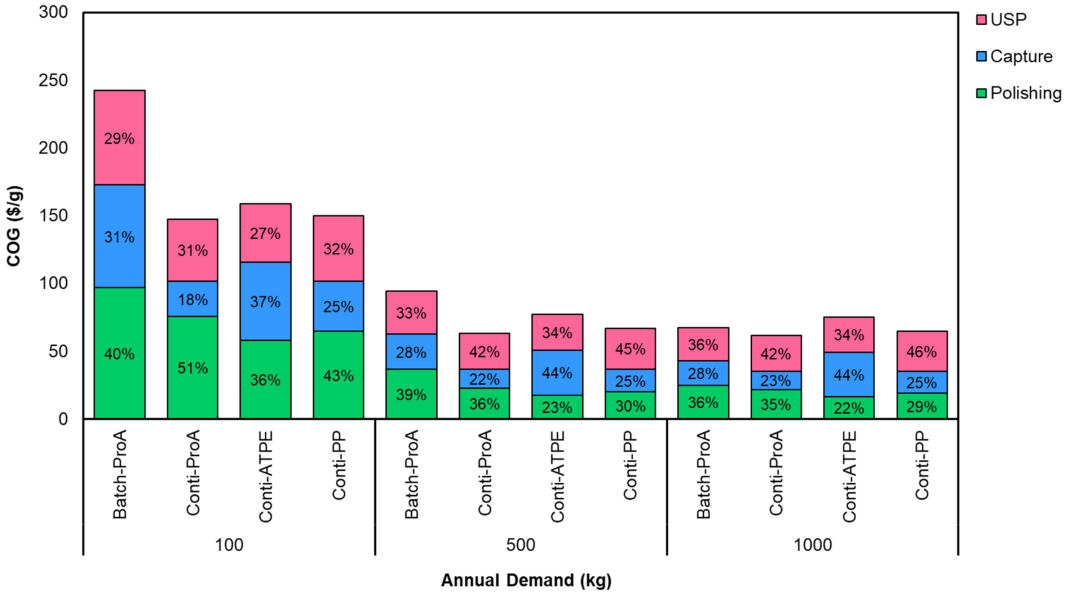


FIGURE 3 Breakdown of (a) COG/g per processing stage and (b) impact of each cost category in the different of four mAb production flowsheets. The COG breakdown on a process stage basis is showed for 100, 500, and 1000 kg/year commercial scales, while the contribution of each process stage in each cost category is shown for the 500 kg/year scale. USP and polishing steps are fixed among continuous flowsheets, while the capture stage comprises different unit operations.

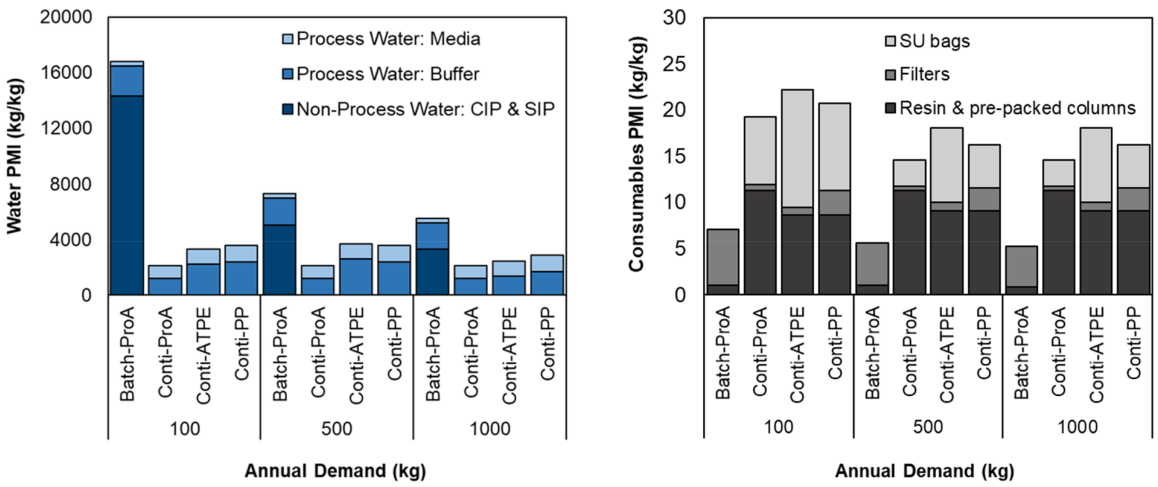


FIGURE 4 Water and consumables process mass intensity (PMI) breakdown for four mAb production flowsheets at 100, 500, and 1000 kg/year commercial scales. The water and consumables PMIs include the complete production train liquid and solid waste, respectively. The consumables PMI is based on the total weight of individual disposable material (SU bags, filters, resin, and prepacked columns). The weight of each material was found in literature or given by suppliers. SU bags include both bioreactor bags and buffer hold bags.

strategy. Although the continuous options have a lower water PMI than the batch flowsheet, the continuous column-free options fare worse than the continuous ProA option. The higher water PMIs of Conti-ATPE and Conti-PP compared with Conti-ProA can be attributed to the higher media consumption and diafiltration buffers.

In contrast to the water PMI trends, the consumables PMI in Figure 4b was 4 to 5-fold higher in continuous mode (e.g. 500 kg/year consumables PMI: 5 kg/kg Batch-ProA, 15 kg/kg Conti-ProA, 18 kg/kg Conti-ATPE, and 16 kg/kg Conti-PP). However, the order of magnitude is negligible compared with the lower liquid waste. On the consumables front, as expected from the cost analysis, Conti-ATPE resulted in a higher consumables PMI (SU bags and membranes). Regarding Conti-PP, contrary to the consumables cost savings compared with Conti-ProA, the consumables PMI in Conti-PP was in fact higher than the column-based option. This comes from the higher usage of filters and SU bags that outweigh the reduction in consumables weight (kg) from the absence of Pro-A prepacked columns. Overall, Conti-ATPE presents a water PMI and a consumables PMI approximately 70% and 20% higher than Conti-ProA, respectively, and Conti-PP presents a water PMI and

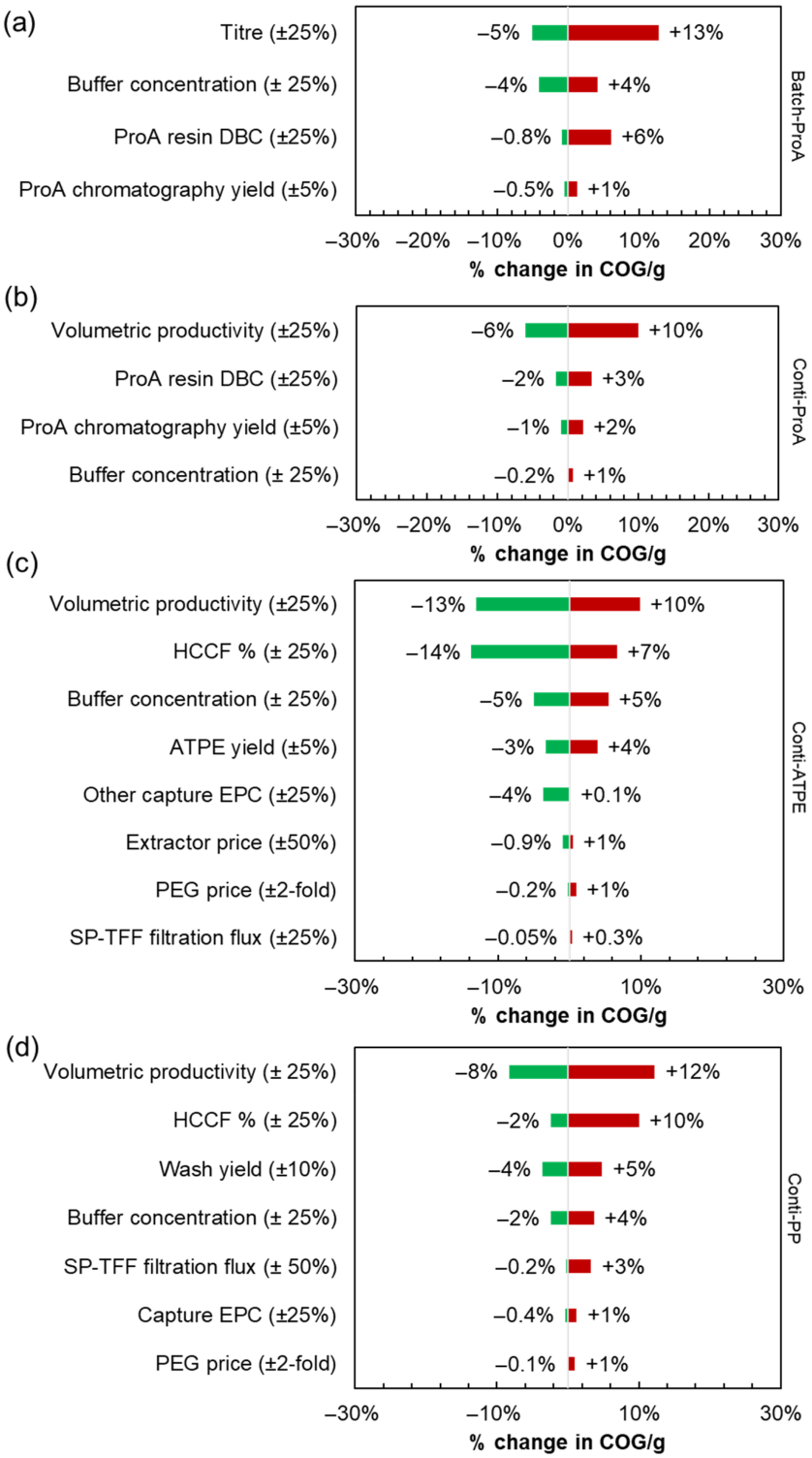


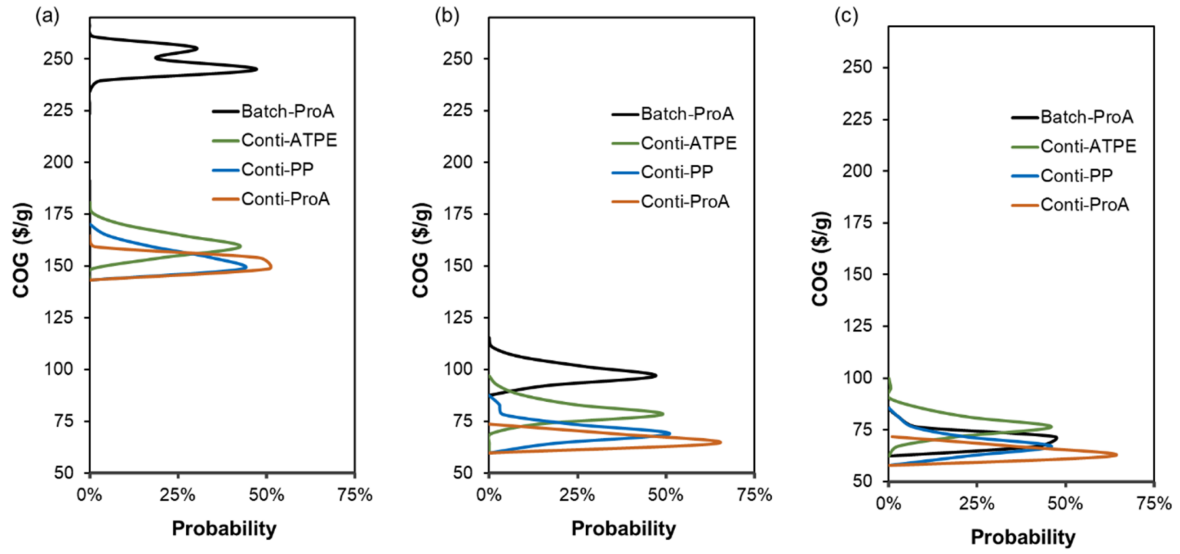
FIGURE 5 Sensitivity analysis of COG/g showing the effect of process parameters variation on (a) Batch-ProA, (b) Conti-ProA, (c) Conti-ATPE, or (d) Conti-PP mAb production flowsheets, at 500 kg/year scale. The percentage differences are relative to the COG/g in the base case.

consumables PMI approximately 60% and 10% higher than Conti-ProA, respectively.

The water and consumables PMI values are within the range of values reported in the literature for continuous mAb flowsheets[14,23,29,30] and suggest that continuous and single-use technologies can be key enablers for improving environmental impact in terms of overall PMI.

3.3 | Robustness evaluation with uncertainty analysis

In every large-scale bioprocess there are inherent uncertainties; thus, it is important to identify the key sources for technical deviations and account for them in the cost model to generate representative results. While the economic and environmental advantages of pursuing Conti-



(d)

Demand (kg)	100				500				1000			
Flowsheet	Batch-ProA	Conti-ProA	Conti-ATPE	Conti-PP	Batch-ProA	Conti-ProA	Conti-ATPE	Conti-PP	Batch-ProA	Conti-ProA	Conti-ATPE	Conti-PP
Deterministic COG ($/g)	242	148	159	150	94	63	77	67	67	61	75	65
eCOG ($/g)	246	149	158	151	96	64	78	68	68	62	75	66
Standard deviation	5.2	2.5	4.7	4.3	4.2	1.8	4.2	3.9	3.2	1.7	4.3	4.2
p-value (Batch-ProA)	N.A	<0.001	<0.001	<0.001	N.A	<0.001	<0.001	<0.001	N.A	<0.001	<0.001	<0.001
p-value (Conti-ProA)	<0.001	N.A	<0.001	<0.001	<0.001	N.A	<0.001	<0.001	<0.001	N.A	<0.001	<0.001
p(COG ≤ $eCOG_{ProA}$)	0%	49%	1%	31%	0%	52%	0%	13%	1%	51%	0%	12%

FIGURE 6 COG/g probability distribution plots under manufacturing uncertainty at (a) 100 kg/year, (b) 500 kg/year, and (c) 1000 kg/year production scales. (d) Statistical data on COG/g for the competing technologies under process variability across demands. The *p*-values were computed using a two-tailed homoscedastic *t*-test with an alpha value of 0.05; *p*-values below this value indicate a significant difference. *p*-value (Batch-ProA) and *p*-value (Conti-ProA) refer to the values when the COG distributions from each flowsheet were compared with that of Batch-ProA and Conti-ProA, respectively. eCOG = expected COG.

ProA strategy were highlighted during the deterministic cost comparison, a stochastic analysis enabled the evaluation of different scenarios under process variability. The first step was to conduct a sensitivity analysis by changing some critical process parameters in the column-based and column-free capture flowsheets to understand, which factors had the largest influence on mAb production costs and to identify the major risks or benefits for production in terms of process changes. The selected ranges presented were discussed with academic and industrial partners, so the analysis could fairly represent the best and worst technical parameters found for each one of the technologies.

The results of the sensitivity analysis are illustrated in the tornado diagrams in Figure 5. The diagrams illustrate that the key COG driver is the titer (in Batch-ProA) and volumetric productivity (in Conti-ProA/ATPE/PP) in cell culture. Lower titers/productivities than expected resulted in higher USP costs as larger or more bioreactors were required to meet the demand. This had a knock-on impact on total reagent costs, dominated by CIP buffer costs in Batch-ProA (58%) and media costs in the continuous strategies (>75%). On the other hand, working with increased volumetric productivities and more concentrated HCCF would benefit specially the column-free alternatives, as lower perfusion volumes would require a smaller DSP. Figure 5c,d shows that the HCCF percentage was the second parameter with the largest impact on the COG in both column-free capture alternatives. The HCCF% in the ATPE or PP systems' composition determined the dilution of the broth and the volume handled in the following steps. Therefore, changes at this level had a large impact on the equipment investment (associated with the indirect costs), consumables and reagents costs. In Conti-ATPE, an increase from 18% to 25% of perfusion liquid in the ATPE system (HCCF%) could decrease the final COG/g by more than 10% at medium and large scales, demonstrating the benefits of a lower product dilution on the cost-effectiveness of liquid extraction for mAb capture.

The concentration of buffers was also a factor to be considered when looking at process changes that could reduce costs in column-free strategies. As the bag containers dominate the equipment costs for the capture sequence in Conti-ATPE and Conti-PP, using buffer concentrates and inline dilution would bring savings in the final COG. Other changes in parameters, such as the reagents' price, filtration fluxes, or other equipment price (besides capture EPC) had a lower

TABLE 2 Multi-criteria decision-making summary of weights, ratings and overall aggregate weighted scores.

Criteria category, k	Criteria, i	Rank	Weight, E_i	Normalized weight, w_i	Rating value, x_{ij}			Standardized rating, r_{ij}			Weighted category score, y_{jk}			Overall aggregate score, S_j ($R_{eco} = 0.8$, $R_{env} = 0.1$, $R_{op} = 0.1$)		
					Conti ProA	Conti ATPE	Conti PP	Conti ProA	Conti ATPE	Conti PP	Conti ProA	Conti ATPE	Conti PP	Conti ProA	Conti ATPE	Conti PP
Economic (500 Kg)	Cost of goods ($/g)	1	1	0.5	63	77	67	100	0	75	100	0	85	97	5	75
	Fixed capital investment ($)	1	1	0.5	25 M	27 M	25 M	100	0	96						
Environmental (500 Kg)	Water PMI (kg/kg)	1	1	0.5	2156	3684	3566	100	0	54	100	0	31			
	Consumables PMI (kg/kg)	1	1	0.5	15	18	16	100	0	8						
Operational	Robustness	1	5	0.33	4.4	4.0	2.5	84	75	38	72	54	36			
	Ease of scale-up	2	4	0.27	3.4	4.7	3.8	60	92	69						
	Ease of validation	3	3	0.20	3.9	2.7	2.0	72	42	25						
	Ease of operation	4	2	0.13	3.3	2.7	3.5	56	42	63						
	Ease of installation	5	1	0.07	2.9	4.0	3.8	47	75	69						

Note: Rank of 1 indicates most important. For the operational metrics, a rating value of 5/5 represents the best outcome.

impact on the final COG/g; thus, they are not considered to portray significant risks for the process. The individual parameter changes that resulted in greater than a 5% change in COG were selected and integrated into the uncertainty analysis using Monte Carlo simulations, where the process mass output was fixed (100, 500, and 1000 kg/year) and the facility was resized for each iteration to reflect the consequences of different starting assumptions.

Accounting for key uncertainties in the batch and continuous processes with a stochastic analysis enables the robustness of the options to be determined as well as the likelihood of meeting certain COG/g threshold values. The results of the stochastic Monte Carlo analysis are depicted in the COG frequency distributions in Figure 6 with an embedded table of key statistics. The figure shows that Conti-ProA presented the most robust alternative across demands compared with the batch and continuous column-free options as indicated by its narrower distribution and lowest standard deviation and hence risk. It had also the lowest expected cost and the differences in COG distributions were found to be statistically significant, as indicated by all p-values being below 0.05 (embedded table). Of the column-free options, Conti-PP had the higher probability of matching Conti-ProA expected COG values, with a likelihood ranging from 10% to 30% across scales (embedded table). Bimodal distributions, with peaks occurring at different COG values for the same alternative and scale, were observed for Batch-ProA at 100 kg/year and Conti-PP at 500 kg/year, when there was a jump in bioreactor scale due to low titer or productivity, respectively. Apart from this scenario, uncertainties in titers, process yields, HCCF% or binding capacities did not represent major shifts in the most likely COG/g for each flowsheet (peaks from stochastic distributions are within 5% of costs attained in the deterministic analysis, as shown in the embedded table).

3.4 | Multi-criteria decision-making

MCDM analysis was used to reconcile economic, environmental, and operational criteria and identify the most advantageous continuous strategy considering all perspectives. While the economic (COG/g and FCI) and environmental (water and consumables PMI) criteria were directly obtained as model outputs, the qualitative criteria (e.g., ease of scale-up, ease of validation) were derived from survey responses from academia and industry experts with experience in affinity chromatography, liquid–liquid extraction and precipitation used in mAb capture. Table 2 summarizes the key values used in the MCDM to compute the overall aggregate scores for each flowsheet (ProA chromatography, ATPE, and PP), including the criteria weights, standardized ratings, and weighted category scores. From the list of qualitative operational criteria, robustness was the most important metric, while ease of installation ranked last, based on the survey responses. The radar chart in Figure 7a shows all standardized rating values for each criteria for each flowsheet and it was used to simplify the visualization of the preferred flowsheet at each criteria. As expected, Conti-ProA scored the highest in all economic and environmental criteria. Moreover, it had the maximum score in two out of five operational criteria,

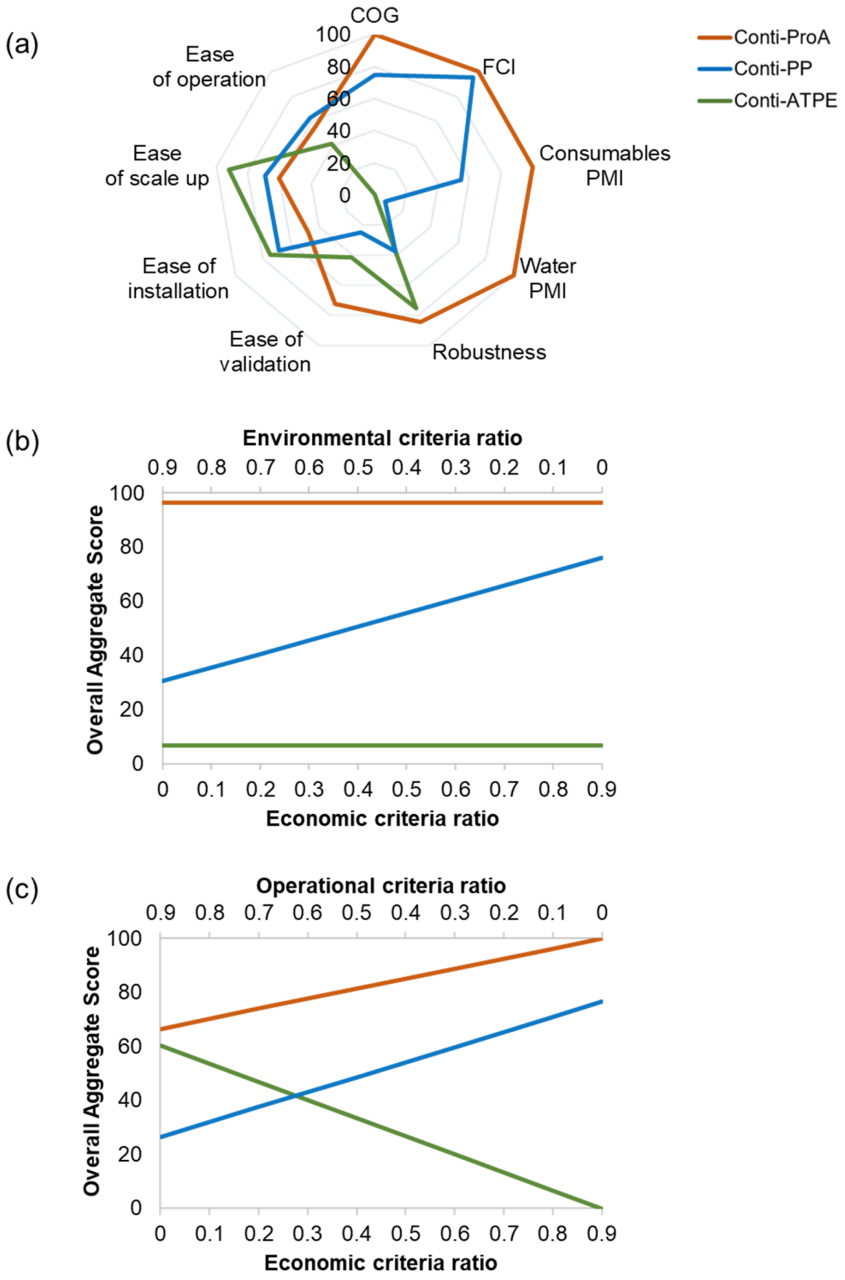


FIGURE 7 (a) Rating values of Conti-ProA, Conti-ATPE, and Conti-PP flowsheets for each economic (cost of good—COG; fixed capital investment—FCI), environmental (consumables PMI; water PMI), and operational (robustness, ease of operation, scale-up, installation, and validation) criteria. (b, c) Effect of the economic, operational, and environmental criteria combination ratios on the overall aggregate scores when the operational attribute ratio is constant at 10% (b) and when the environmental attribute ratio is constant at 10% (c). All graphs are generated for a mAb demand of 500 kg/year.

including robustness, the most important metric. Conti-ATPE had very poor scores across all quantitative metrics due to its high COG/g, equipment cost, consumable usage, and water consumption; however, its operational feasibility was reasonably high according to the qualitative scores given by experts. Conti-PP scored well in the economic criteria, whereas, operational-wise, it only showed high scores for the two least important criteria (ease of operation and installation).

To reconcile the competing outputs, the overall aggregate score was generated for each flowsheet across different combination ratios of the economic, environmental, and operational categories (Figure 7b,c). The resulting sensitivity spider plots illustrate how the ranking of the alternative continuous options changes depending on user priorities. The figures clearly illustrate that Conti-ProA was the preferred continuous strategy irrespective of the relative importance of the economic, environmental, or operational category scores. The ranking between the remaining column-free options of Conti-PP and Conti-ATPE depended on the weightings of the categories. When economic and environmental performance were prioritized, Conti-PP was preferred over Conti-ATPE for all combinations of these two categories (Figure 7b). However, when operational performance was brought into the picture as a key priority (Figure 7c) and weighed against economic savings, then a switch point occurred where the operational category was twice as important as the economic category ($R_{op} = 0.6, R_{eco} = 0.3$). When the operational benefits dominated in the final score above this threshold ($R_{op} > 0.6$) then Conti-ATPE become the preferred column-free option over Conti-PP.

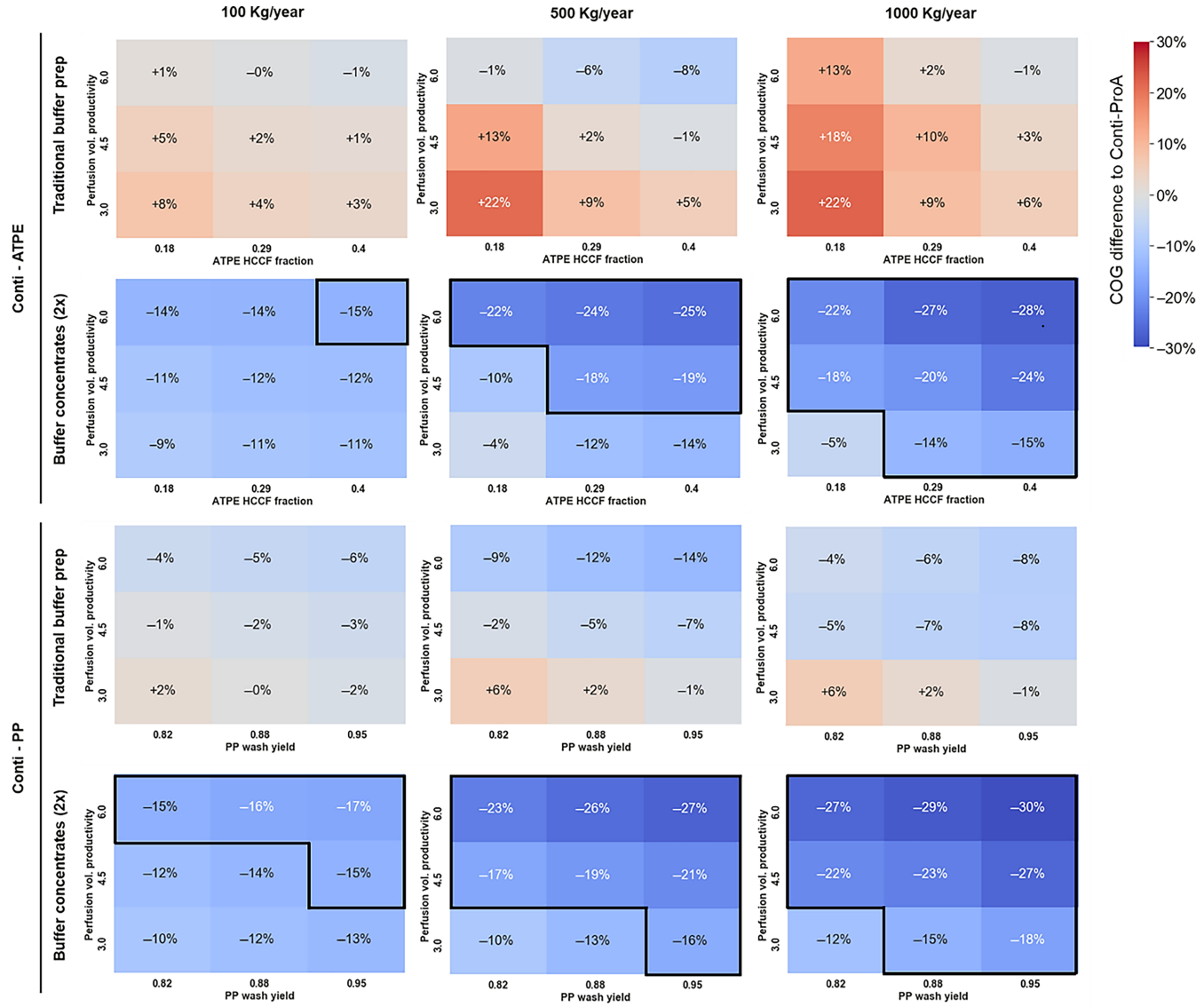


FIGURE 8 Heat maps showing the COG difference for Conti-ATPE and Conti-PP relative to Conti-ProA as a function of the perfusion volumetric productivity versus either the HCCF% fraction for Conti-ATPE or the wash yield for Conti-PP. The target analysis is shown for scenarios using traditional buffer preparation as well as buffer concentrates. * indicates the base case scenario. The area within the solid black line indicates the conditions at which Conti-ATPE and Conti-PP present ≥15% COG/g savings compared with Conti-ProA.

3.5 | Target analysis

The earlier COG analysis in this article showed that the continuous mAb facilities modeled with column-free capture technologies did not offer lower manufacturing costs compared with the column-based option (Conti-ProA). This section determines the cost reductions required for column-free alternatives to achieve a target COG saving threshold of at least 15% compared with the continuous flowsheet with ProA capture to justify the process change. The ATPE and PP process changes implemented were based on the parameters that had the highest impact on COG/g savings in the sensitivity analysis, namely the perfusion volumetric productivity with either the ATPE HCCF% or the PP wash yield.

Figure 8 displays the target analysis as a matrix of heatmaps across scales and buffer preparation methods to determine the windows of operation where parallel improvements in ATPE and PP flowsheets result in COG savings that meet the target threshold of 15% (highlighted by the region within the black solid lines).

With traditional buffer preparation, the target COG saving was not achieved regardless of the combination of parameters in the column-free flowsheets. Also, in Conti-ATPE, possible increases in volumetric productivity or HCCF fraction rarely led to COG values matching Conti-ProA COG. On the other hand, in Conti-PP, as the base-case COG is already very similar to Conti-ProA, changes in these process parameters led to scenarios offering modest savings over ProA chromatography.

The implementation of inline dilution of buffers (2-fold buffer concentrates) across all options conferred a strong advantage particularly for both Conti-ATPE and Conti-PP. The target COG saving could be reached for a broad combination of parameters in Conti-ATPE and Conti-PP. The window of feasible combinations meeting the target increased as scales increased due to the larger contribution of consumables and reagents costs to the total COG at higher scales and the ability of the process changes to minimize material consumption.

The attractiveness of Conti-ATPE and Conti-PP will depend on the improvement of multiple process parameters to levels that may be beyond the current best cases found in literature. The usage of buffer concentrates is becoming more commonplace and improved perfusion volumetric productivities may be envisioned for the near future. Also, the implementation of a simple pre-concentration step before capture has been already discussed with partners and it would resemble the benefits of having higher volumetric productivities, as working with a more concentrated HCCF would lead to smaller volumes during DSP. However, increasing the HCCF percentage in the ATPE/PP systems without compromising capture performance and achieving higher step yields would entail further studies on the technical optimisation of ATPE and precipitation as capture technologies applied to mAbs.

4 | CONCLUSION

This article presented the extent of capabilities configured in a process economics model that enabled the comparison of different mAb production flowsheets from economic, environmental, and robustness perspectives. The simulation tool built in Python was used to design batch and continuous facilities and provided an in-depth evaluation of the trade-offs associated to ProA chromatography, ATPE and product precipitation as mAb capture steps across production scales. The cost drivers for each scenario were highlighted and determined that the implementation of continuous manufacturing was preferable over batch, especially at lower scales, and that the broth dilution in ATPE and higher media consumption in PP could favor the selection of ProA as capture step in continuous mAb processing. Although there was an increase in consumables usage in continuous mode, the environmental analysis showed that the water savings found over batch would decrease the overall environmental burden associated with continuous mAb production. The MCDM analysis presented higher aggregate scores for continuous mAb processing with column-based capture across scenarios with different weightings for economic, operational, and environmental performance. The target analysis showed that ATPE and PP could provide lower COG than ProA if buffer concentrates are implemented and if the cell culture volumetric productivity, the HCCF% in the ATPE system and precipitates wash yields were maximized altogether. The added value of such a simulation framework was revealed through the assessment of different technologies, flowsheets and scenarios, as these are critical during process development and decision-making on future facility designs in the biopharmaceutical sector.

AUTHOR CONTRIBUTIONS

Catarina P. G. Neves: Conceptualization; data curation; formal analysis; investigation; methodology; software; visualization; writing – original draft; writing – review and editing. **Jonathan L. Coffman:** Conceptualization; resources; validation. **Suzanne S. Farid:** Conceptualization; funding acquisition; project administration; resources; supervision; validation; visualization; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supporting information of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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