

Article

Side-by-Side Economic Process Model for the Comparison and Evaluation of Magnetic Bead-Based Processes and Legacy Process for the Manufacturing of Monoclonal Antibodies

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Abstract: This study models two alternative downstream processes based on magnetic separation with the objective of understanding the economic feasibility of these processes compared to the traditional mAb process. The key focus lies in the economic understanding of the cell harvest and capture steps in the models. Here, the models revealed that integrating cell removal and product capture in a single operation is the main factor driving the unified productivity between USP and the magnetic bead-based processes. This results in significant economic benefits, such as savings in both the cost of goods per gram of mAb and fixed costs, as well as increasing annual facility output. The predicted savings potential approaches 38% for COGs, 17% for capital investment, and 40% for annual facility output. For mammalian cell-based manufacturing, the magnetic separation-based DSP provides a highly valuable option due to its integration of several individual unit operations compared to the traditional process both in reducing process time and cost and accommodating higher demands.

Keywords: magnetic beads; economic modelling; magnetic separation; process integration; mAb manufacturing; high cell density



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1. Introduction

In modern medicine, monoclonal antibodies (mAbs), recognised by their distinguished Y-shape [1], play an important role in the treatment of various serious diseases, such as oncological and immunological diseases [2]. The use of mAbs as a therapeutic modality has skyrocketed in recent decades, with more than 100 approved mAbs for various diseases [3,4]. To support the growing demand for mAbs and other antibody-related therapeutics, the manufacturing processes are constantly on trial for improvements [5–7], facilitating the delivery of larger quantities at a lower cost [8]. This is the case particularly for biosimilars as they compete without patent protection and at lower revenue per each dose. In recent years, many manufacturers have shown interest in disruptive technologies and conducted studies with the aim of supporting decisions based on changes in their legacy process platforms. Impressive increases have been achieved in mammalian cell culture for mAbs, with product titres claimed today at up to 10 g/L for fed batch [9–11] and perfusion-based cultures pushing productivity even further [12–16]. A common denominator of these improvements is a higher cell mass and an increase in volumetric mAb productivity [12–17]. This subsequently impacts the clarification, capture, and purification process steps, e.g., with more challenging cell removal and a demand for increased load

capacity in chromatography steps [6,18]. Hence, DSP needs to evolve in sync with USP productivity by, for example, limiting yield loss by means of directly capturing the product from the cell broth.

In bioprocessing, decisional tools, based on different strategies, for instance, single attribute or multiple attribute tools, have been developed and applied to address the feasibility of different manufacturing strategies [19–24]. Process economics modelling allows for a detailed analysis of different process indicators, such as process throughput, COGs, capital investment, and environmental impact [5,25]. Additionally, it allows for exploring different production scales and productivities, determining improvements to increase facility output, and reducing COGs and capital investment. It is essential to benchmark new technologies and applications against existing ones to assess their competitiveness and to objectively find their operational window.

The current legacy DSP for mAb production is centred around column chromatography-based methods [7,26,27]. This means, in turn, that one relies on clarified culture broth since solid particles can cause major interferences during such operations [18]. Steps dedicated to the clarification of cell culture broth become inevitable for the legacy process. Centrifugation and depth filtration steps are commonly used to provide sufficient solid removal for the following column-based product capture [18,26,28–30], in which mAb manufacturing is almost always Protein A affinity chromatography [26,28–30].

In recent years, alternative methodologies [28,29,31] have been suggested to cope with some of the new large-scale processing challenges. More recent studies have investigated magnetic separation, which, despite its existence for decades [32–34], has long been overlooked [17,31,35–39]. Magnetic separation can cope with high cell density in the load stream, is gentle towards cells, leading to the slower release of impurities, and is rapid [17,31,36,38–40]. As a result, it offers the opportunity to eliminate cell clarification steps and to shorten DSP batch time accordingly [17,31,39,41]. Shorter batch time can lead to lower cost associated with the occupancy time of a facility, alternatively to more batch runs in a given time, and thus a higher output from the facility.

This study looks at the challenges imposed by cell culture intensification on DSP steps at the industrial scale and evaluates the cost-competitiveness and feasibility of different DSP scenarios to cope with these challenges. Here, a mAb manufacturing campaign of a total of 60 batches in one year was modelled based on two magnetic separation processes compared to the legacy process, which is based on a different methodology, namely centrifugation and filtration for cell removal and column chromatography for the target capture. This study focuses on the economic understanding and implication the two magnetic separation-based processes provided in comparison to the legacy process. Moreover, the objectives were to understand the impact of the integrated cell removal and target capture, provided by the magnetic separation, regarding the major cost drivers and their contribution to the cost of goods per gram of product (USD/g) per process step and to facility output (kg/y).

The comparison of a manufacturing process based on magnetic separation to the traditional column-based process offers an in-depth understanding of the trade-offs between different technologies and allows for the identification of the key parameters that influence the selection of the best manufacturing strategy.

2. Materials and Methods

2.1. Decisional Tool

Dept. of Biochemical Engineering, University College London, has a long history in the development and application of decisional tools in bioprocessing to assess the feasibility of alternative manufacturing strategies [19,42]. A process economics model for mAbs, originally developed at UCL, was extended by Decisional Point Ltd. (Henley-on-Thames, UK) to incorporate MAGic™ Bioprocessing's (Uppsala, Sweden) MAGicBeads and MAGicAccio System Process to perform an in-depth process economics analysis. The model performs a series of mass balance and process design calculations to determine the size of each unit operation, the consumption of resources, the utilisation of the manufacturing

facility, the capital investment, and the COGs/g. The process flowsheet forms the core for the process economics model and is used as the basis for all assumptions and comparisons.

In this study, three different process flows are used (see Figure 1). To achieve comparability, the three processes are each modelled with the same process input assumptions. The USP is modelled with 4 bioreactors operated in staggered mode. The bioreactor volume was determined at 500 L with two different titres at 6.6 g/L and 10.8 g/L. The titres were obtained from intensified fed-batch cell cultures used in previous magnetic separations [17]. Each reactor is modelled with 15 batches per year, and each batch has a run time of 29 days, including the seed expansion, with 3 times over 5 days, resulting in 15 days and a production bioreactor of 14 days. These are typical durations for the cultivation of CHO cells via a fed-batch bioreactor. Hence, the model calculates 60 total batches per one year (335 days of operational time) to maximize unit operation and facility utilisation. Aside from the given set points for the USP, the starting point for the tool is the mass balance calculation to assess the correct sizing of manufacturing equipment. The equipment is divided into core equipment like columns, filter holders, and centrifuges and peripheral equipment like buffer and product hold tanks. The design calculations, based on the fixed input values for the different unit operations, were performed at UCL based on previous work [19,43]. The tool's output, process economics, includes fixed capital investment (USD M), facility output (kg/y), and COGs/g (USD/g). Fixed capital investment is estimated via the Lang factor method [44–46]. The Lang factor method is a well-known method used in chemical engineering, described by Peter and Timmerhaus [46] for the determination of the capital expenditure to build a chemical manufacturing plant based on equipment purchase costs. This was adapted by Novais et al. [45] for bioprocessing. Facility output represents a 12-month campaign output in considering the number of batches (15 per reactor, 60 total batches), the bioreactor output per batch (3.3 kg–5.4 kg), and the yield loss during the chain of DSP unit operations. Lastly, COGs/g of mAb depict labour costs, indirect cost, and direct costs. Direct costs mainly capture the costs for reagents (e.g., culture media or buffers) and consumables (e.g., chromatography resin or filter capsules). The indirect costs combine all costs commonly associated with operating a manufacturing facility, such as insurance, maintenance, depreciation, and taxes. Labour costs account not only for the operators at each operational step but also for supervisors, management, and quality assurance (QA) and quality control (QC).

With the input values of harvest volume and product concentration as given, the dependent parameters, like the volume of beads, number of separator units, and duration of the different magnetic separation phases, are calculated.

The bead or resin volume is calculated as follows:

$$V_{beads} = \frac{Mass_{inlet} [g]}{DBC [\frac{g}{L}]} \quad (1)$$

Here, V_{beads} is the volume of beads per litre (magnetic resins or packed column volume) required to bind the mass of the target molecule ($Mass_{inlet}$) in relation to the binding capacity (DBC) of the corresponding resin. Furthermore, calculations were introduced to accommodate for the new requirements allocated to the magnetic separation. Specifically, the number of magnetic separator units is as follows:

$$n = \frac{V_{beads} [L]}{Capacity\ Separator\ Unit [L]} \quad (2)$$

where n describes the number of separators as the relation of total magnetic bead volume to the maximum capacity of beads a single separator holds. In addition to the number of separators, the different phase durations are important and are calculated as shown below:

$$t_{load} = \frac{V_{broth} [L] + V_{beads} [L]}{n * Q_{load} [\frac{L}{h}]} \quad (3)$$

$$t_{wash} = \left(\frac{BV_{wash} * V_{beads} \left[\frac{L}{h} \right]}{n * Q_{wash} \left[\frac{L}{h} \right]} \right) * wash\ cycles \quad (4)$$

$$t_{elution} = \left(\frac{BV_{elution} * V_{beads} \left[\frac{L}{h} \right]}{n * Q_{elution} \left[\frac{L}{h} \right]} + t_{incubation} [h] \right) * elution\ cycles \quad (5)$$

Herein, t_{load} , t_{wash} , and $t_{elution}$ in hours describe the duration of the different phases. t_{load} is calculated from the sum of the cell broth volume (V_{broth}) and beads volume (V_{beads}), divided by the product of the number of separators (n) and the load flow rate (Q_{load}). The wash duration is described through the unitless wash buffer (BV_{wash}) and the beads volume (V_{beads}), the number of separators (n), Q_{wash} the wash flow rate, and the number of wash cycles. t_{clean} and t_{store} are calculated like t_{wash} . The elution time is calculated similarly to the wash duration but with an added time for the incubation of loaded beads in the elution buffer ($t_{incubation}$). The numerical values are shown in Appendix A, Table A1. The process economics modelling tool is used in combination with a data sheet containing all the assumptions for the process (Table 1) from the starting input values of the cell harvest and concentration over to the step yield, solid removal with its associated losses and, equipment and material costs. The output from the simulation tool is exported into MS Excel [19].

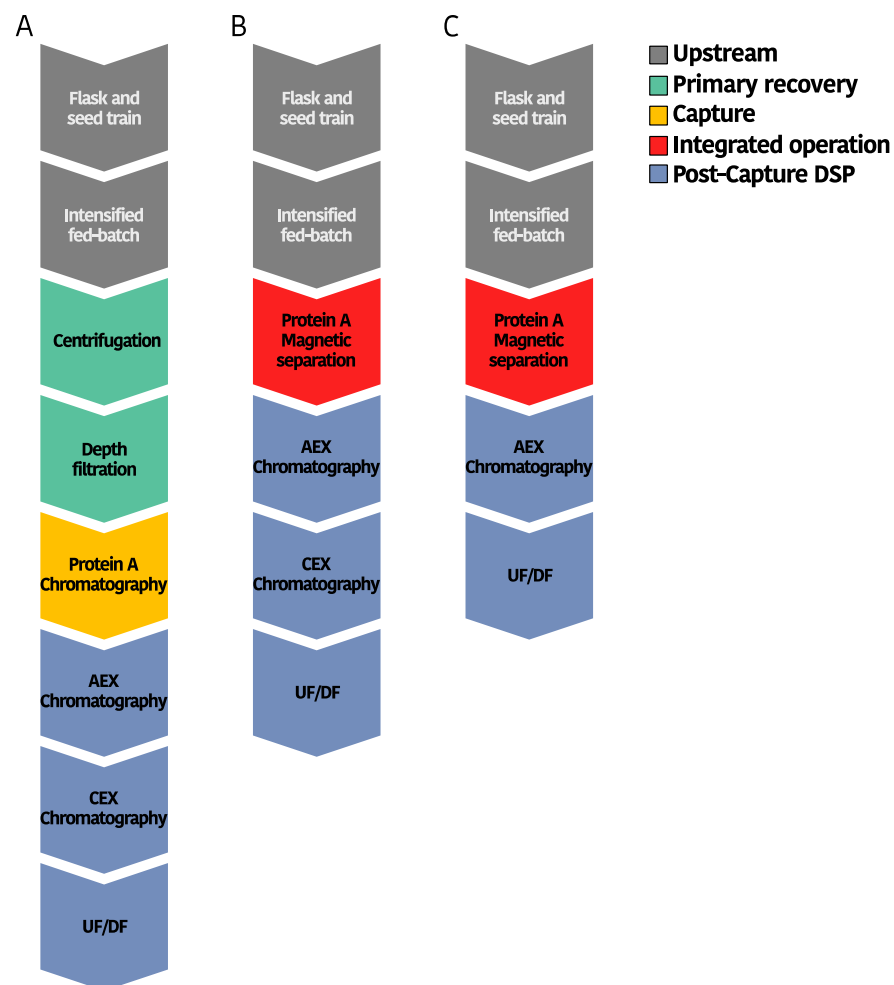


Figure 1. Flowsheet for the three modelled processes, (A) CHROM, (B) MAG-2, and (C) MAG-1, containing the relevant process operational steps. Note that post-capture DSP also includes two steps not depicted here that are related to viral safety (VI—low pH virus inactivation and VF—virus filtration).

Table 1. Key assumptions and parameters used in the model. The parameters are based on experimental data for the MAG-2 and MAG-1 process, and for the CHROM process, the data are obtained from a database.

Key Assumptions	CHROM		MAG-2		MAG-1	
Harvest concentration (g/L)	6.6	10.8	6.6	10.8	6.6	10.8
Bioreactor scale (L)	500	500	500	500	500	500
USP:DSP ratio	4:1	4:1	4:1	4:1	4:1	4:1
No. of reactors	4	4	4	4	4	4
No. of batches per reactor	15	15	15	15	15	15
Total batch time (d)	31	31	31	31	31	31
Time seed train (d)	15	15	15	15	15	15
Time production culture (d)	14	14	14	14	14	14
DSP time (d)	2	2	2	2	2	2
No. of total batches	60	60	60	60	60	60
Resin lifespan (#cycles)	100	100	100	100	100	100
Load challenge (g/L)			identical			
Step yield (%)			identical			
Protein A LRV			identical			
Biomass content	10%	18%	10%	18%	10%	18%
Solid carry-over	5%	5%				
Centrifugation yield	92%	82%				
Depth F. capacity (L/sqm)	250	150				
Depth F. flux (LMH)	200	150				
Depth F. yield	95%	90%				
Overall process yield	62%	54%	72%	72%	75%	75%

2.2. Manufacturing Scenarios

The process economics analysis presented here is based on three different DSP scenarios. Namely, the traditional process (CHROM), a magnetic beads-based process with two polishing steps as in the CHROM process (MAG-2), and a similar process with only one polishing step (MAG-1) (see Figure 1). The three flowsheets were compared assuming a 500 L stirred tank production bioreactor scale at two different mAb titres of 6.6 g/L and 10.8 g/L. The process economics model is supported by experimental data obtained from different mAb capture experiments using magnetic separation [17,39]. To minimise the idle time of the DSP line, the facility was modelled with 4 production bioreactors, each with a batch time of 29 days, including a seed train and production bioreactor, operated in staggered mode, resulting in a harvest every second day to feed a single DSP train with a batch time of two days each. After the 4th production bioreactor batch is a 10-day break followed by a new turnaround. In this layout, 60 batches are performed over one year.

The traditional mAb platform process is modelled with the same input values as the magnetic separations processes to ensure a fair comparison among the different scenarios. The upstream process design is identical for all scenarios (Table 1). For the traditional process (Figure 1), harvesting of the production bioreactor is performed. Firstly, we assume a disc-stack centrifuge with varying yields depending on the process biomass (Table 1). Secondly, we assume an additional filtration step due to the 5% carry-over of solids (Table 1). In addition, we assume a yield decline due to the different biomass (Table 1). After the clarification of the cell culture broth, a Protein A capture step follows. Next in line for the traditional process is a low pH virus inactivation step and two chromatography polishing steps (AEX and CEX). A virus filtration step (VF) followed by buffer exchange and concentration (UF/DF) concludes the process sequence. This common layout of a mAb manufacturing process is the basis for the economic analysis.

Two possible new process strategies are identified when implementing magnetic separation instead of the traditional Protein A capture column [17,39]. MAG-2 (Figure 1) deviates significantly in the midstream operation and the capture step but still uses two polishing chromatography steps: instead of the centrifugation, depth filtration, and Protein A column chromatography capture, a single magnetic separation unit operation is

implemented. The magnetic separation step copes with non-clarified cell broth, combining the unit operations of clarification and capture and eliminating the need for several individual ones. The following steps are identical to the traditional process. The improvement over MAG-2 that MAG-1 (Figure 1) implements is the elimination of one of the two chromatography polishing steps (CEX). This process scenario assumes the low mechanical stress observed at the bench and pilot scale, resulting in low Host Cell Protein (HCP) levels after the magnetic separation can justify the removal of one of the polishing chromatography steps [17,39] and still meet the product quality specifications.

3. Results

Addressing the bottlenecks in the current mAb manufacturing process is essential to accommodate the increasing need and sustainability for antibody-based therapeutics. Improvements in the culturing process have led to higher cells densities and volumetric productivities. However, if the following DSP steps cannot handle the improvements coming from the USP, the gained achievements are immediately lost. Therefore, process improvements should always follow a holistic approach and avoid focusing on the optimisation of a single process step. This study investigates holistic approaches to tackle the challenges imposed by cell culture intensification on the DSP.

3.1. Breakdown of Cost of Goods

Figure 2 presents the process-related cost contribution of the different unit operations per produced amount of mAb. These unit operations can further be categorised into USP (grey), primary recovery (yellow), capture (orange for traditional chromatography and red for magnetic separation), and post-capture DSP (blue). Here, in Figure 2, three downstream scenarios (CHROM, MAG-2, and MAG-1) are shown. These three scenarios are fed with the exact same USP input (Table 1) at two titres of 6.6 g/L and 10.8 g/L (Figure 2). In the case of the presented model that is reflected in the four production bioreactors, each of 500 L, operated in staggered mode, this results in a total of 60 batches per year. Notably, the DSP trains differ in terms of type and number of DSP operations that follow (Figure 1 and Table 1). The cost contribution shown in Figure 2 is based on our assumptions and free available data, and the exact numbers are subject to more factors such as quantity discount; therefore, the individual cost contribution can significantly deviate between different manufactures. However, the trends seen in Figure 2 are a good indicator. Looking at the total process-associated cost, a clear trend is seen with a reduction in the COGs/g for both MAG processes in comparison to the CHROM process. For the 6.6 g/L feed, 21.6 and 44.6% reduction is achieved for the MAG-2 and MAG-1 process, respectively. For the 10.8 g/L feed, the reduction is even greater, with 29 and 50.6% for the MAG-2 and MAG-1 processes, respectively (Figure 2). This is not a surprising trend as all three processes (CHROM, MAG-2, and MAG-1) deviate in terms of process steps, and integrating multiple single steps results in overall less steps and step-related losses. Here, the integrated mAb capture directly from the cell broth plays its great strength by combining three individual steps into a single-unit operation. This becomes obvious, especially for the highest single cost contributor, the Protein A-related step (Figure 2 orange, red). If individually scrutinised, the traditional process (CHROM) provides the lowest cost contribution of all three processes for that step, with 35.2 USD/g and 28.1 USD/g for the 6.6 g/L and the 10.8 g/L processes, respectively (Figure 2, orange). Both magnetic-based processes (MAG-2 and MAG-1) show a higher cost contribution for the Protein A-associated step (Figure 2, red). From the economic perspective, the magnetic step is not favouring a decision towards a process change, but the step integration it brings in the form of direct capture has a huge positive economic impact. When viewing the combined cost contribution of the Protein A-associated step and the clarification steps (Figure 2, yellow and orange), then the CHROM process shows a significant higher economic burden compared to the two magnetic-based processes (MAG-2 and MAG-1). Here, the process-associated COGs/g for the CHROM process almost doubles to 66.6 USD/g and 49.3 USD/g for the 6.6 g/L and 10.8 g/L feed, respectively. This,

again, shows the benefit of the step integration of multiple unit operations into a single-unit operation. In addition to the above-shown benefits of step integration, the COGs/g breakdown shows even more interesting insides. Excluding clarification and capture, the USP is a cost factor which is worth investigating. The USP part of the process (Figure 2, grey), which accounts for the seed train and production culture, is identical for all processes. Despite being identical for all three DSPs, the process-related cost contribution for the USP is decreasing in both magnetic-based processes by up to 20 and up to 30% for the 6.6 g/L and 10.8 g/L feed, respectively. It is notable that the overall cost of the USP remains the same, but putting the costs in relation to the total mAb output of the process lowers the cost contribution significantly. Importantly, this effect is also seen for the other unit operations. The polishing steps of AEX and CEX (Figure 2, blue), virus inactivation and virus filtration (dark blue and light blue), and UF/DF (very light blue) are operated at the same total costs, but due to the improved process yield of the magnetic bead-based processes, these costs are divided by a larger mAb quantity. Hence, the single-unit operation also results in lower COGs/g of mAbs. Notably, in addition to the MAG-1 process, due to the increased step reduction, only one polishing step, an even more favourable COGs/g breakdown, is seen. In essence, the magnetic-based processes due to step integration and step reduction increase the overall process yield, leading to a higher output at the same costs, which in turn improves the COGs/g for the different unit operations.

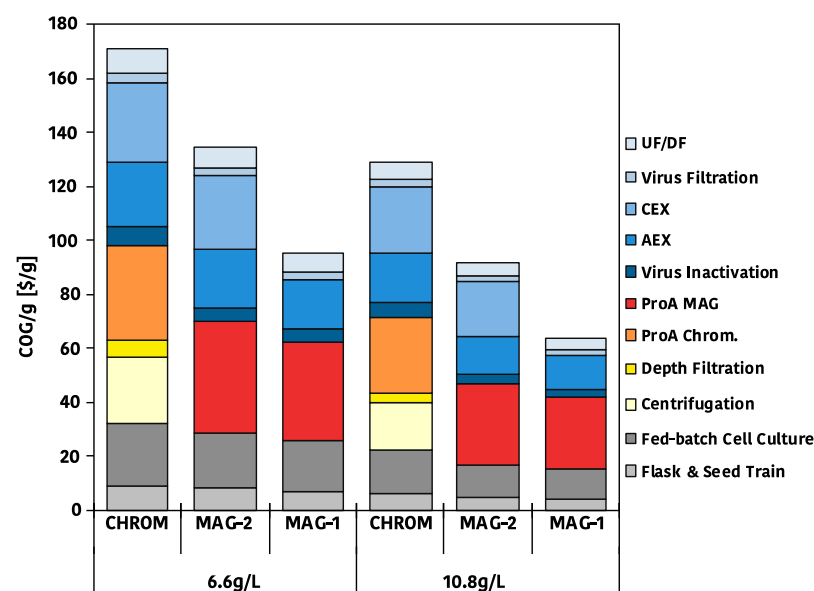


Figure 2. Step-related COG/g for the respective unit operations and three processes CHROM, MAG-2, and MAG-1 at two different titres.

Table 2 shows the annual facility output as well as the facility output change and the changes in COGs/g and fixed capital investment relative to the traditional process (CHROM) across the processes at 500 L scale and product titres, as mentioned above. Firstly, a reduction by 5% and 17% in fixed capital costs is seen for both magnetic processes compared to the traditional process. These fixed capital costs here are only accounting for the equipment cost. Hence, the process with the lowest number of steps and equipment (MAG-1) provides the highest savings, while the MAG-2 process provides savings only at the clarification stage. Furthermore, the magnetic bead-based processes show a reduction in COGs/g ranging from 18 to 29% for the 6.6 g/L feed and 27 to 38% for the 10.8 g/L feed. Here, again, as all processes deal with the identical input from the USP, the step integration and reduction achieved by the magnetic bead-based processes are the reason for these significant improvements. This fact also resembles in the process output increase. Here, the magnetic processes show improvements of 15 to 20% and 34 to 40% for the MAG-1 and

MAG-2 processes, respectively, at the modelled titres and a scale of 500 L. The reduction in unit operations with less process-related yield losses results in an overall higher total process yield, which leads to all the significant process improvements.

Table 2. Output process performance and performance change for the three modelled processes at two different product titres.

	CHROM	6.6 g/L MAG-2	MAG-1	CHROM	10.8 g/L MAG-2	MAG-1
Output (kg/60 batches)	123	142	148	174	233	243
Output change ¹	0%	15%	20%	0%	34%	40%
FCI change ¹	0%	−5%	−17%	0%	−5%	−17%
Total COG/g change ¹	0%	−18%	−29%	0%	−27%	−38%

¹ all changes are in relation to the CHROM process.

3.2. Annual Output Analysis

Complementing the presented investigation, the annual output was modelled at different mAb concentrations and scales. The model assumes that all processes have the same USP with an identical number of batches per reactor (15) and a culture time of 29 days including the seed expansion (15 days) and production bioreactor (14 days). The USP is operated in a staggered mode with four production bioreactors resulting in 60 total batches for one year identical to the COGs analysis. The processes, however, deviate in terms of scale, mAb titre, cell density, DSP performance, and, hence, the overall process yield (Table 3).

Table 3. Modelled annual output at scale of 500 L in relation to harvest titre, USP output, overall process output, and overall process yield.

	Harvest Concentration (g/L)	USP Output (kg/y)	Process Output (kg/y)	Process Yield (%)
CHROM	3	90	59	65
	6	180	112	62
	9	270	154	57
	12	360	188	52
	15	450	227	50
MAG-2	3	90	65	72
	6	180	129	72
	9	270	194	72
	12	360	259	72
	15	450	323	72
MAG-1	3	90	67	75
	6	180	135	75
	9	270	202	75
	12	360	269	75
	15	450	337	75

The annual output is displayed in relation to the USP titre of 3 g/L, 6 g/L, 9 g/L, 12 g/L, and 15 g/L, which translates to the cell density and at different production scales of 200 L (Figure 3A), 500 L (Figure 3B), 1000 L (Figure 3C), and 2000 L (Figure 3D). Of particular interest is the annual output at the 500 L scale (Figure 3B) as it matches the scale used in the COGs/g breakdown. Figure 3B includes the traditional process and the two magnetic bead-based processes (MAG-2 and MAG-1), providing an insight into the annual output in relation to the USP titre. For the traditional process (CHROM), the output curve (blue line) describes a linear behaviour at low titres; hence, with low cell densities but with raising titres and cell density, the output does not increase linearly. The curve depicts that the higher cell mass needed to produce the higher titres lowers the annual output of the

CHROM process. Here, the bottleneck of the cell clarification really shows, with higher cell mass, especially the centrifugation step, suffering from poor yield [47,48]. This is especially shown in the process output numbers. At a titre of 3 g/L, the traditional process provides a USP output of 90 kg/y (Table 3). After the cell clarification and DSP, an annual process output of 59 kg/y of mAb is modelled (Table 3 and Figure 3B). A relatively good efficiency is reached. However, with a 5-fold increase in titre to 15 g/L, the annual process output only increases 3.85-fold, i.e., a 68 kg lower output than expected at first glance, resulting in a total process yield of 50% compared to the 65% seen at low cell densities (Table 3). This shows how drastically the higher cell densities effect the cell clarification step and result in a less efficient process and significant product loss compared to the gained USP output. The MAG-2 and MAG-1 processes do not suffer from cell density-related yield loss during the clarification. Hence, both magnetic bead-based processes show a linear relation between the productivity of the UPS and the annual process output (organ and grey line). For example, the MAG-2 process, at the same scale (500 L) and titre (3 g/L) as the CHROM process, with the same USP output of 90 kg, provides an overall process output of 65 kg/y (Table 3 and Figure 3B). Even at the lower range of titre and cell density, the process output is already slightly higher compared to the traditional process, which has an output of 59 kg/y (Table 3 and Figure 3B). Looking at the same 5-fold change in titre to 15 g/L, a process output of 323 kg/y (Table 3 and Figure 3B) is achieved, i.e., there is no increased loss of yield due to increased titre in connection with the higher cell density for the MAG-2 process. This is reflected also in the overall process yield, which remains constant at the different titres and does not decline as for the CHROM process (Table 3). Lastly, the MAG-1 process at a titre of 3 g/L has an annual mAb output of 67 kg/y (Table 3). When operated at the same 5-fold titre increase (15g/L), MAG-1 provides a yearly process output of 337 kg/y (Table 3 and Figure 3B). Again, MAG-1 also does not show an increased loss of yield in conjunction with the titre and cell density, proven by the constant process yield of 75% (Table 3). However, it should be noted that the process outputs and process yield are similar to the MAG-2 process and indicate a low impact of removing one polishing step on the overall process output. Comparing MAG-1 and MAG-2, only a gain of 3% is achieved in the MAG-1 process (Figure 3B). This gain becomes slightly higher at a high titre and high cell densities but remains low with just 4%, as is seen by the low offset between the grey and orange line (Figure 3B). The modelled annual output highlights that no increased loss of yield due to increased titre in connection with the higher cell density is seen for either of the magnetic bead-based processes. The behaviour described for Figure 3B is similar for the other scales as well (Figure 3A,C,D) but with distinct differences in the annual output due to the reactor's size in the modelled facility.

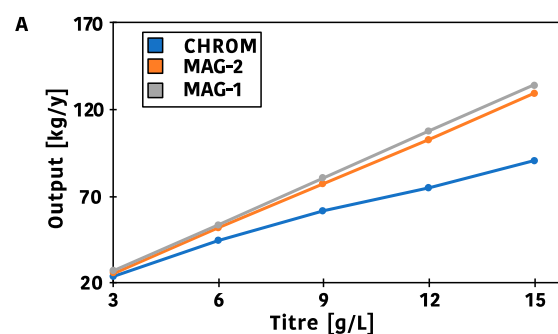


Figure 3. Cont.

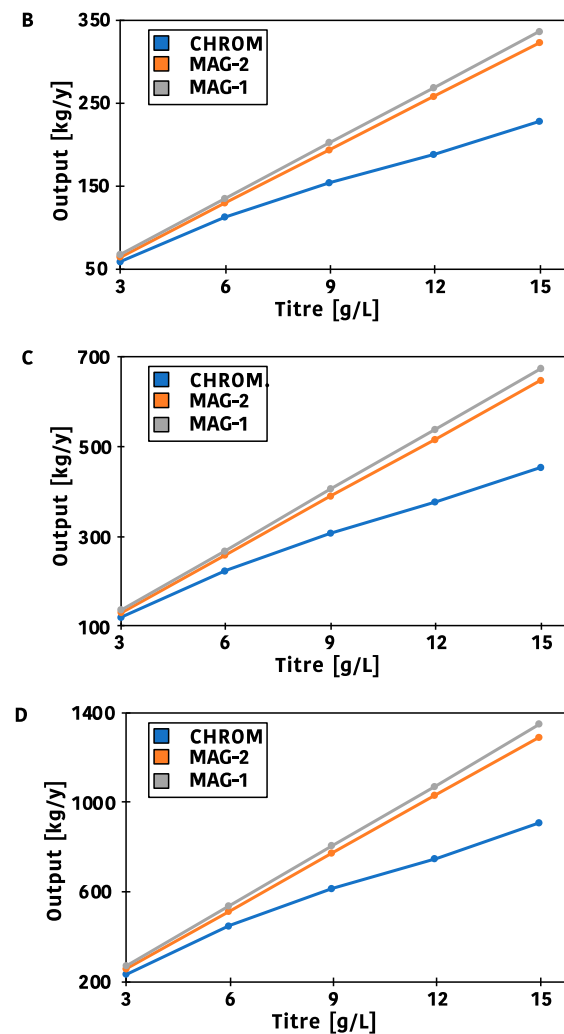


Figure 3. Modelled annual process throughput based on different titres at 200 L (A), 500 L (B), 1000 L (C), and 2000 L (D) production scale for monoclonal antibodies.

4. Discussion

This study provides an in-depth analysis of the key indicators for the economic assessment of downstream operations for the manufacturing of monoclonal antibodies comparing the traditional process (CHROM) and two magnetic separation-based processes (MAG-2 and MAG-1). Here, the critical focus is to understand the economic viability of the two magnetic separation-based processes in comparison to the legacy process, especially with regard to process intensification, such as high titre and associated high cell density.

Comparing the three modelled processes, the magnetic-based processes (MAG-2 and MAG-1) outperform the traditional process (CHROM) (see Table 2 and Figure 2). Both MAG processes show significant gains in terms of productivity and savings in COGs/g. The model highlights the impact of integrating individual process steps into a single-unit operation when processing high-cell-density cell broth. Magnetic separation combines the step of cell clarification and affinity capture to enable the integration into a single step. Due to the integration, the overall process yield improves. Hence, the overall process productivity increases in comparison to the traditional process, with more product remaining from the same reactor input. In turn this also effects the COGs/g of every process step. Although the costs for the unit operations are the same for all three processes, except the traditional Protein A step and the magnetic separation, relative to the overall mAbs output, the COGs decline.

The step integration is also reflected in the saved fixed capital investments. Naturally removed steps and associated equipment result in lower investment costs. However, it needs to be highlighted that the model assumes an empty production plant. Hence, the savings in fixed capital investment are only true for new investments. In reality, equipment can be re-used, leading to a lower investment, which can compensate for the savings shown by the model.

In addition, for process MAG-1, the model assumes the removal of one of the polishing steps (CEX step). CEX removal is based on the low mechanical stress obtained at the bench and pilot scale [17,39], resulting in low Host Cell Protein (HCP) levels after the magnetic separation, which potentially necessitates just one chromatography polishing step. Despite the fewer steps of the MAG-1, the removal of the polishing step does not result in a significant gain in terms of process productivity (Table 2). The integration of cell clarification and capture steps has the highest impact; the yield during the polishing step is commonly very high, and very little product is lost at this step. Therefore, the removal of this step does not significantly improve the overall process economics.

Furthermore, both magnetic processes (MAG-2 and MAG-1) show promising outcomes in the form of the predicted annual output. The predicted process output (Figure 3) and the resulting process yield (Table 3) accentuate the benefits of magnetic separation when it comes to the processing of high-cell-density cell broth. For both MAG processes, the process yield remains constant at the different titres (Table 3), while at the same time the yield reduces for the CHROM process. The model displays the lack of traditional DSP (CHROM) to match the productivity of the USP compared to the two MAG processes. This indicates that the current DSP still presents a major bottleneck in the mAb manufacturing process. In particular, the cell clarification remains a problematic process step. With increasing mAb titres and the associated increasing cell densities, the clarification steps reach their limitation, leading to product loss, insufficient cell removal, and/or prolonged processing time [18,49]. The product loss shows the biggest negative impact on our modelled processes, resulting in the higher cost of goods per gram of antibody and lower productivity as the USP output cannot be translated into the overall process output. The current strategies cannot keep up with the improvement in the upstream part of the process. This loss of productivity at higher mAb concentrations and cell densities needs to be addressed to supply the future demand. On the other hand, magnetic separation unlocks its true potential at higher mAb titres and higher cell densities, e.g., as seen in intensified processes [17,39]. Where centrifugation and filtration can reach their limitations, magnetic separation helps to omit the step-related losses and is mechanically gentle towards the cells, lowering the release of impurities such as HCPs [17,39]. On the contrary, the common clarification steps used in the traditional process provide high mechanical stress towards the cells, which leads to the release of HCPs [50], thus increasing the purification burden.

Lastly, it should be noted that neither of the two MAG processes is superior to the other. The two processes should not be seen as either/or but rather specific to the manufacturing circumstances. The MAG-1 provides great improvements regarding the reduction in COGs/g (Figure 2). Here, the removal of the CEX polishing step is the main reason for the significant reduction in COGs/g. However, the removal of the CEX step might be reasonable from a COGs point of view but does not significantly improve the overall process productivity (Figure 3). Therefore, if specific impurity requirements demand an additional polishing step, MAG-2 might be more adequate as the productivity is similar to the MAG-1 process. All these factors culminate in the superior performance of both magnetic bead-based processes in our model compared to the traditional process for mAb production.

5. Conclusions

Process intensifications on the USP side of mAb manufacturing have pushed the productivity of the USP to exceptional high cell densities and mAb titres. But current clarification technologies can hardly cope with high cell densities, leading to poor yield

and resulting eventually in the loss of productivity for the following DSP. Henceforth, a lot of the gained improvements from the USP are lost in the following steps.

In the presented modelling study, the traditional DSP train for mAb manufacturing, based on cell clarification, compares to two magnetic separation-based DSP trains with integrated clarification and capture to evaluate the feasibility of these DSP scenarios to cope with the challenges imposed by cell culture intensification. Here, magnetic separation-based DSP has been, to our understanding, modelled for the first time in a manufacturing setting.

The model provides a unique insight into how magnetic separation tackles the challenge of high-cell-density cell broth processing for the manufacturing of mAbs. In particular, the step integration of cell clarification and mAb capture enabled by the magnetic separation shows itself to be the key driver for significant process improvements compared to the traditional process. Most impressively is the matched productivity between the USP and magnetic separation-based DSP translating the gain through the whole process.

In conclusion, the economic modelling shows that magnetic separation-based DSP can be highly beneficial for the manufacturing of mAbs. This becomes most apparent when the USP dictates a single non-clarified harvest; here, the magnetic separation is superior towards the traditional processing with a significant gain in output and savings in COGs/g and fixed capital investment.

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Appendix A

Table A1. Numerical values for the durations of the different magnetic separation phases used for the calculation of process time.

	6.6 g/L	10.8 g/L
t_{load} [min]	39	40
t_{wash} [min]	64	102
t_{elution} [min]	13	15

References

1. Le Basle, Y.; Chennell, P.; Tokhadze, N.; Astier, A.; Sautou, V. Physicochemical Stability of Monoclonal Antibodies: A Review. *J. Pharm. Sci.* **2020**, *109*, 169–190. [[CrossRef](#)] [[PubMed](#)]
2. Castelli, M.S.; McGonigle, P.; Hornby, P.J. The pharmacology and therapeutic applications of monoclonal antibodies. *Pharmacol. Res. Perspect.* **2019**, *7*, e00535. [[CrossRef](#)] [[PubMed](#)]
3. Mullard, A. 2020 FDA drug approvals. *Nat. Rev. Drug Discov.* **2021**, *20*, 85–91. [[CrossRef](#)] [[PubMed](#)]
4. Mullard, A. FDA approves 100th monoclonal antibody product. *Nat. Rev. Drug Discov.* **2021**, *20*, 491–495. [[CrossRef](#)] [[PubMed](#)]
5. Farid, S.S. Process economics of industrial monoclonal antibody manufacture. *J. Chromatogr. B* **2007**, *848*, 8–18. [[CrossRef](#)]
6. Singh, N.; Arunkumar, A.; Chollangi, S.; Tan, Z.G.; Borys, M.; Li, Z.J. Clarification technologies for monoclonal antibody manufacturing processes: Current state and future perspectives. *Biotechnol. Bioeng.* **2016**, *113*, 698–716. [[CrossRef](#)]
7. Aldington, S.; Bonnerjea, J. Scale-up of monoclonal antibody purification processes. *J. Chromatogr. B* **2007**, *848*, 64–78. [[CrossRef](#)]
8. Chen, C.; Wong, H.E.; Goudar, C.T. Upstream process intensification and continuous manufacturing. *Curr. Opin. Chem. Eng.* **2018**, *22*, 191–198. [[CrossRef](#)]

9. Handlogten, M.W.; Lee-O'Brien, A.; Roy, G.; Levitskaya, S.V.; Venkat, R.; Singh, S.; Ahuja, S. Intracellular response to process optimization and impact on productivity and product aggregates for a high-titer CHO cell process. *Biotechnol. Bioeng.* **2018**, *115*, 126–138. [\[CrossRef\]](#)
10. Huang, Y.-M.; Hu, W.; Rustandi, E.; Chang, K.; Yusuf-Makagiansar, H.; Ryll, T. Maximizing productivity of CHO cell-based fed-batch culture using chemically defined media conditions and typical manufacturing equipment. *Biotechnol. Prog.* **2010**, *26*, 1400–1410. [\[CrossRef\]](#)
11. Takagi, Y.; Kikuchi, T.; Wada, R.; Omasa, T. The enhancement of antibody concentration and achievement of high cell density CHO cell cultivation by adding nucleoside. *Cytotechnology* **2017**, *69*, 511–521. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Mellahi, K.; Brochu, D.; Gilbert, M.; Perrier, M.; Ansorge, S.; Durocher, Y.; Henry, O. Process intensification for the production of rituximab by an inducible CHO cell line. *Bioprocess Biosyst. Eng.* **2019**, *42*, 711–725. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Pinto, N.D.S.; Napoli, W.N.; Brower, M. Impact of micro and macroporous TFF membranes on product sieving and chromatography loading for perfusion cell culture. *Biotechnol. Bioeng.* **2020**, *117*, 117–124. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Xu, J.; Rehmann, M.S.; Xu, M.; Zheng, S.; Hill, C.; He, Q.; Borys, M.C.; Li, Z.J. Development of an intensified fed-batch production platform with doubled titers using N-1 perfusion seed for cell culture manufacturing. *Bioresour. Bioprocess.* **2020**, *7*, 17. [\[CrossRef\]](#)
15. Xu, J.; Xu, X.; Huang, C.; Angelo, J.; Oliveira, C.L.; Xu, M.; Xu, X.; Temel, D.; Ding, J.; Ghose, S.; et al. Biomanufacturing evolution from conventional to intensified processes for productivity improvement: A case study. *mAbs* **2020**, *12*, 1770669. [\[CrossRef\]](#)
16. Xu, S.; Gavin, J.; Jiang, R.; Chen, H. Bioreactor productivity and media cost comparison for different intensified cell culture processes. *Biotechnol. Prog.* **2017**, *33*, 867–878. [\[CrossRef\]](#)
17. Brechmann, N.A.; Schwarz, H.; Eriksson, P.-O.; Eriksson, K.; Shokri, A.; Chotteau, V. Antibody capture process based on magnetic beads from very high cell density suspension. *Biotechnol. Bioeng.* **2021**, *118*, 3499–3510. [\[CrossRef\]](#)
18. Singh, N.; Pizzelli, K.; Romero, J.K.; Chrostowski, J.; Evangelist, G.; Hamzik, J.; Soice, N.; Cheng, K.S. Clarification of recombinant proteins from high cell density mammalian cell culture systems using new improved depth filters. *Biotechnol. Bioeng.* **2013**, *110*, 1964–1972. [\[CrossRef\]](#)
19. Stamatis, C.; Farid, S.S. Process economics evaluation of cell-free synthesis for the commercial manufacture of antibody drug conjugates. *Biotechnol. J.* **2021**, *16*, 2000238. [\[CrossRef\]](#)
20. Xenopoulos, A. A new, integrated, continuous purification process template for monoclonal antibodies: Process modeling and cost of goods studies. *J. Biotechnol.* **2015**, *213*, 42–53. [\[CrossRef\]](#)
21. Klutz, S.; Holtmann, L.; Lobedann, M.; Schembecker, G. Cost evaluation of antibody production processes in different operation modes. *Chem. Eng. Sci.* **2016**, *141*, 63–74. [\[CrossRef\]](#)
22. Pollock, J.; Coffman, J.; Ho, S.V.; Farid, S.S. Integrated continuous bioprocessing: Economic, operational, and environmental feasibility for clinical and commercial antibody manufacture. *Biotechnol. Prog.* **2017**, *33*, 854–866. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Hummel, J.; Pagkaliwangan, M.; Gjoka, X.; Davidovits, T.; Stock, R.; Ransohoff, T.; Gantier, R.; Schofield, M. Modeling the Downstream Processing of Monoclonal Antibodies Reveals Cost Advantages for Continuous Methods for a Broad Range of Manufacturing Scales. *Biotechnol. J.* **2019**, *14*, 1700665. [\[CrossRef\]](#)
24. George, E.; Titchener-Hooker, N.J.; Farid, S.S. A multi-criteria decision-making framework for the selection of strategies for acquiring biopharmaceutical manufacturing capacity. *Comput. Chem. Eng.* **2007**, *31*, 889–901. [\[CrossRef\]](#)
25. Farid, S.S.; Washbrook, J.; Titchener-Hooker, N.J. Decision-Support Tool for Assessing Biomanufacturing Strategies under Uncertainty: Stainless Steel versus Disposable Equipment for Clinical Trial Material Preparation. *Biotechnol. Prog.* **2005**, *21*, 486–497. [\[CrossRef\]](#)
26. Kelley, B. Industrialization of mAb production technology: The bioprocessing industry at a crossroads. *mAbs* **2009**, *1*, 443–452. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Thömmes, J.; Etzel, M. Alternatives to Chromatographic Separations. *Biotechnol. Prog.* **2007**, *23*, 42–45. [\[CrossRef\]](#)
28. Kelley, B. Very Large Scale Monoclonal Antibody Purification: The Case for Conventional Unit Operations. *Biotechnol. Prog.* **2007**, *23*, 995–1008. [\[CrossRef\]](#)
29. Low, D.; O'Leary, R.; Pujar, N.S. Future of antibody purification. *J. Chromatogr. B* **2007**, *848*, 48–63. [\[CrossRef\]](#)
30. Shukla, A.A.; Wolfe, L.S.; Mostafa, S.S.; Norman, C. Evolving trends in mAb production processes. *Bioeng. Transl. Med.* **2017**, *2*, 58–69. [\[CrossRef\]](#)
31. Ebeler, M.; Lind, O.; Norrman, N.; Palmgren, R.; Franzreb, M. One-step integrated clarification and purification of a monoclonal antibody using Protein A Mag Sepharose beads and a cGMP-compliant high-gradient magnetic separator. *New Biotechnol.* **2018**, *42*, 48–55. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Whitesides, G.M.; Kazlauskas, R.J.; Josephson, L. Magnetic separations in biotechnology. *Trends Biotechnol.* **1983**, *1*, 144–148. [\[CrossRef\]](#)
33. Dunnill, P.; Lilly, M.D. Purification of enzymes using magnetic bio-affinity materials. *Biotechnol. Bioeng.* **1974**, *16*, 987–990. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Ugelstad, J.; Kaggerud, K.H.; Hansen, F.K.; Berge, A. Absorption of low molecular weight compounds in aqueous dispersions of polymer-oligomer particles, 2. A two step swelling process of polymer particles giving an enormous increase in absorption capacity. *Makromol. Chem.* **1979**, *180*, 737–744. [\[CrossRef\]](#)
35. Ebeler, M.; Pilgram, F.; Wolz, K.; Grim, G.; Franzreb, M. Magnetic Separation on a New Level: Characterization and Performance Prediction of a cGMP Compliant “Rotor-Stator” High-Gradient Magnetic Separator. *Biotechnol. J.* **2018**, *13*, 1700448. [\[CrossRef\]](#)

36. Holschuh, K.; Schwämmle, A. Preparative purification of antibodies with protein A—An alternative to conventional chromatography. *J. Magn. Magn. Mater.* **2005**, *293*, 345–348. [\[CrossRef\]](#)
37. Fraga García, P.; Brammen, M.; Wolf, M.; Reinlein, S.; Freiherr von Roman, M.; Berensmeier, S. High-gradient magnetic separation for technical scale protein recovery using low cost magnetic nanoparticles. *Sep. Purif. Technol.* **2015**, *150*, 29–36. [\[CrossRef\]](#)
38. Hubbuch, J.J.; Thomas, O.R.T. High-gradient magnetic affinity separation of trypsin from porcine pancreatin. *Biotechnol. Bioeng.* **2002**, *79*, 301–313. [\[CrossRef\]](#)
39. Brechmann, N.A.; Eriksson, P.-O.; Eriksson, K.; Oscarsson, S.; Buijs, J.; Shokri, A.; Hjälml, G.; Chotteau, V. Pilot-scale process for magnetic bead purification of antibodies directly from non-clarified CHO cell culture. *Biotechnol. Prog.* **2019**, *35*, e2775. [\[CrossRef\]](#)
40. Safarik, I.; Safarikova, M. Magnetic techniques for the isolation and purification of proteins and peptides. *BioMagn. Res. Technol.* **2004**, *2*, 7. [\[CrossRef\]](#)
41. Ebeler, M.; Pilgram, F.; Wellhöfer, T.; Frankenfeld, K.; Franzreb, M. First comprehensive view on a magnetic separation based protein purification processes: From process development to cleaning validation of a GMP-ready magnetic separator. *Eng. Life Sci.* **2019**, *19*, 591–601. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Mahal, H.; Branton, H.; Farid, S.S. End-to-end continuous bioprocessing: Impact on facility design, cost of goods, and cost of development for monoclonal antibodies. *Biotechnol. Bioeng.* **2021**, *118*, 3468–3485. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Farid, S.S.; Washbrook, J.; Titchener-Hooker, N.J. Modelling biopharmaceutical manufacture: Design and implementation of SimBiopharma. *Comput. Chem. Eng.* **2007**, *31*, 1141–1158. [\[CrossRef\]](#)
44. Lang, H.J. Simplified approach to preliminary cost estimates. *Chem. Eng.* **1948**, *55*, 112–113.
45. Novais, J.L.; Titchener-Hooker, N.J.; Hoare, M. Economic comparison between conventional and disposables-based technology for the production of biopharmaceuticals. *Biotechnol. Bioeng.* **2001**, *75*, 143–153. [\[CrossRef\]](#)
46. Peters, M.S.; Timmerhaus, K.D.; West, R.E. *Plant Design and Economics for Chemical Engineers*; McGraw-Hill: New York, NY, USA, 2003; Volume 4.
47. Gupta, A.; Amara, J.P.; Gousseinov, E.; Cacace, B. Chapter 6—Recent advances in harvest clarification for antibodies and related products. In *Approaches to the Purification, Analysis and Characterization of Antibody-Based Therapeutics*; Matte, A., Ed.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 117–136.
48. Hundley, R.; Königsson, S. Development of a single-use, hermetic centrifuge system. *BioProcess Int.* **2020**, *18*, 26–34.
49. Popova, D.; Stonier, A.; Pain, D.; Titchener-Hooker, N.J.; Farid, S.S. Integrated economic and experimental framework for screening of primary recovery technologies for high cell density CHO cultures. *Biotechnol. J.* **2016**, *11*, 899–909. [\[CrossRef\]](#)
50. Pohlscheidt, M.; Lieser, G.; Bertermann, B.-E.; Eberhardt, H.; Herter, S.; Tebbe, H. At Scale Analysis and Optimization of a Cell Culture Harvest Process—Mitigating Risk and Optimizing Performance. *Am. Pharm. Rev.* **2013**, *16*.

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