Process economic simulation for scalable production of adenovirus

This application note discloses process economic modelling of a modern start-to-finish adenovirus production process. The novel process was compared with a reference process in both stainless steel and single-use configurations across various production scales and scenarios. The process based on modern tools and technologies was generally shown to be the most cost-efficient option of the investigated alternatives. Process configurations using single-use equipment wherever possible were also shown to be more cost-effective than corresponding configurations using primarily stainless-steel equipment.

Introduction

Viral vectors, such as adenovirus, adeno-associated virus, and lentivirus, have been shown to have great potential for both vaccine and therapeutic applications such as cell and gene therapy, and are likely to become important features of a future biopharmaceutical landscape. As such, it is important to build an understanding of how viral vector production can be designed to be both technically and economically feasible as well as for the product to meet regulatory requirements.

A start-to-finish process for production of adenoviruses using modern tools and technologies has previously been described (1). Technical feasibility was demonstrated, and the performance of the process was benchmarked against a reference process using more traditional technology alternatives (2). This work investigates the process economic performance of the two processes. An overview of the novel process as well as the reference process is given in Figure 1.

Methods

Process economic model

Process economic modelling was performed using the BioSolve™ process economic simulation tool (BioPharm services), and costs per batch and per viral dose were compared between process scales of 50, 200, and 500 L, for titers ranging between 1×10^{13} and 9×10^{13} virus particles (vp)/L. Table 1 summarizes the different production scenarios investigated in this study, and how input parameters were adjusted to simulate them.

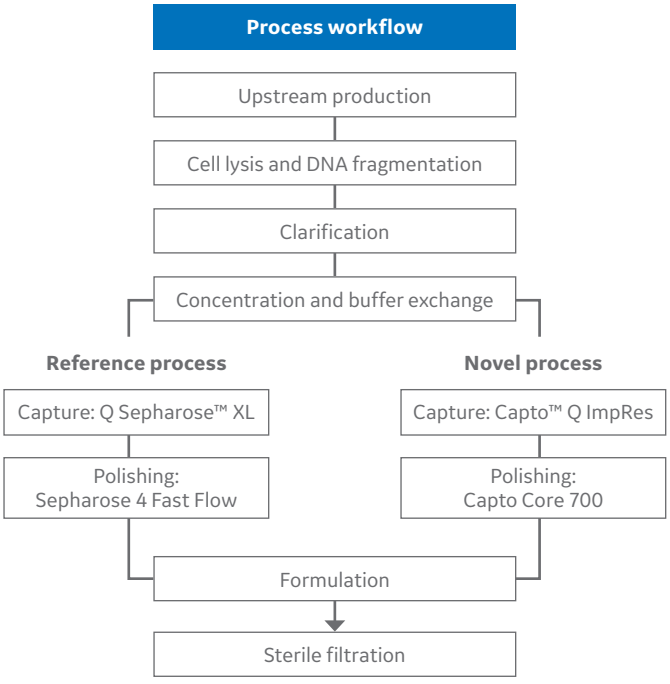

Fig 1. Process outline for the novel process as well as for the reference process for adenovirus production.

General assumptions

General assumptions are summarized in Table 2 for the model and in Table 3 for the most central differences between the two processes. As process recovery has a major impact on cost per dose, the same step recoveries were used in both processes, which enabled a more simple and unbiased comparison.

To account for differences between stainless steel and single-use equipment, the time and personnel requirements associated with activities at the start and end of campaigns were included in each unit operation (such as column packing and cleaning verification). The specific numbers used in the modelling are based on in-house experience in combination with customer input (4). For all non-chromatography unit operations, containing one or more pieces of stainless steel equipment, activities related to end of campaign cleaning and cleaning verification were estimated to 8 h using 1 full-time equivalent (FTE), divided by the number of batches per campaign.

Table 1. Summary of the different production scenarios for adenovirus production investigated in the study

Production scenario	Number of batches per campaign	Number of campaigns per year	BioSolve parameter settings
Single-product facility: commercial production of one product for a large market.	As many as possible	1	Campaign length: 12 months
Multi-product facility: production in a multi-product plant for a smaller market/late stage clinical studies.	3	As many as possible	Estimated number of batches per 12 months at 50 L/200 L/500 L scales: – Single-use equipment = 56/47/41 – Stainless steel equipment = 45/40/35
Single-batch production: production of batches for early phase clinical trials.	1	1	Estimated number of batches per 12 months: 1

Table 2. General assumptions for process economic simulation for adenovirus production

Parameter	Assumption
Dose size	10^{11} vp/dose (3)
Depreciation period for capital investment	8 years
Campaign change-over time	4 days for single-use processes, 7 days otherwise (4)
Target capacity utilization	80%
Number of installed bioreactors	2 (run in staggered mode)
Buffer/medium preparation strategy	Per unit operation (as opposed to per batch or per sub-batch)
Lifetime of chromatography resins*	Sepharose 4 Fast Flow: 200 cycles Q Sepharose XL: 100 cycles Capto Q ImpRes: 100 cycles Capto Core 700: 50 cycles
Lifetime of prepacked ReadyToProcess™ columns	Same as resins packed in user-packed columns†
Yearly maintenance duration	21 days/year
Yearly validation time	14 days/year
Threshold volume for single-use mixers and containers	1000 L
Use stainless steel above threshold	No (i.e., volumes larger than threshold are split into several single-use vessels instead of using one large stainless-steel vessel).
Use of floor area estimate to estimate capital investment	No
Target number of cycles in chromatography steps	3
Process recovery	48%‡

* Based on experimental results. The modelling does not consider that traditional columns can be repacked if the packed bed fails before the resin needs to be replaced. The same recovery was used for both processes.

† The validity of this assumption might vary from process to process, and has not been tested experimentally in this case.

‡ The same recovery was used for both processes.

Table 3. Key settings for selected unit operations

Unit operation	Chromatography resins	Parameter	Setting
Capture chromatography	Q Sepharose XL	Binding capacity	2.50×10^{14} vp/L resin
	Capto Q ImpRes	Binding capacity	5.62×10^{14} vp/L resin
Capture chromatography	Q Sepharose XL	Elution pool volume	0.5 column volumes (CV)
	Capto Q ImpRes	Elution pool volume	1 CV
Polishing chromatography	Sepharose 4 Fast Flow	Capacity	0.15 CV
	Capto Core 700	Capacity	15 CV

Cost categories

BioSolve enables breakdown of the output of each simulation into components based on cost category. The categories discussed in this work are defined as follows:

- Capital: refers to the depreciation of the capital investment for the facility, including the equipment.
- Buffers and media: cover the direct cost of all solutions used during the process.
- Other material costs: cover all the indirect material costs, such as those related to QC tests and out-of-process cleaning-in-place (CIP) procedures.
- Cost of resins and disposable columns: the cost per batch considers how much the resin or disposable column is used before it is disposed.
- Consumables: all non-resin consumables, mainly constituting filters, flow kits, bags, and tubing.
- Labor cost: relates to both running the process and performing associated activities (e.g., column packing, cleaning, and quality control/quality assurance [QC/QA]).
- Other indirect costs: relate to the facility (e.g., utilities, insurance, and waste management)

The following factors were not included in the modelling:

- Cost and time related to installation, operation, and performance qualifications (IQ/OQ/PQ).
- Inoculum and seed train considerations before the first seed bioreactor.
- Failure of packed beds before the complete use of resin lifetime, which would require re-packing of conventional columns or replacement of disposable columns.
- Minor hardware such as scales and tube welders.

Results and discussion

In this process economic simulation, the novel adenovirus production process is compared with a reference process, differing in choice of resins for the virus capture and polishing steps. In addition, single-use and stainless steel process configurations were compared.

Although little or no impact on the batch cost, process step recovery will be a main contributor to the cost per dose. Hence, one must be certain that a difference in recovery is statistically significant before incorporating it into the modelling. Unfortunately, current methods for determination of adenovirus titer are associated with a non-negligible amount of variation (1). To avoid biasing the comparison of the different processes, recovery was set to the same fixed values for both the novel and the reference process. As the overall process recovery was the same for all scenarios (48%), the number of doses produced per year in any scenario depends entirely on the scale and the annual number of batches.

Figure 2 shows how capacity and elution volume of the capture step interact with the capacity of the polishing step. Even though the numbers are based on single-use process configurations, stainless steel configurations follow the same pattern. Although the beneficial cost/L of Sepharose 4 Fast Flow, the low sample load volume allowed for this resin in size exclusion chromatography (SEC) applications (0.15 of column volumes/cycle) will require larger column volumes compared with Capto Core 700 that allows a larger sample load volume (15 column volumes/cycle) to enable processing of the same sample amount. The sample volume to be processed in the polishing step is determined by the binding capacity and collected elution volume of the capture step. Consequently, a low-capacity resin such as Q Sepharose XL will require a larger column size and thus generates a larger elution volume compared with a high-capacity resin such as Capto Q ImpRes for processing of the same sample amount.

Figure 3 shows the cost breakdown by cost category for different production scales at a titer of 5×10^{13} vp/L. It becomes apparent that the cost of Benzonase™ used in the nuclease treatment step emerges as an important factor with increasing bioreactor volume. At 50 L culture scale, this cost is small in comparison with many other cost categories. At 500 L culture scale, however, the Benzonase cost can represent as much as 15% to 20% of the entire batch cost. If possible, is seems sensible to optimize the process to reduce the required amount of Benzonase.

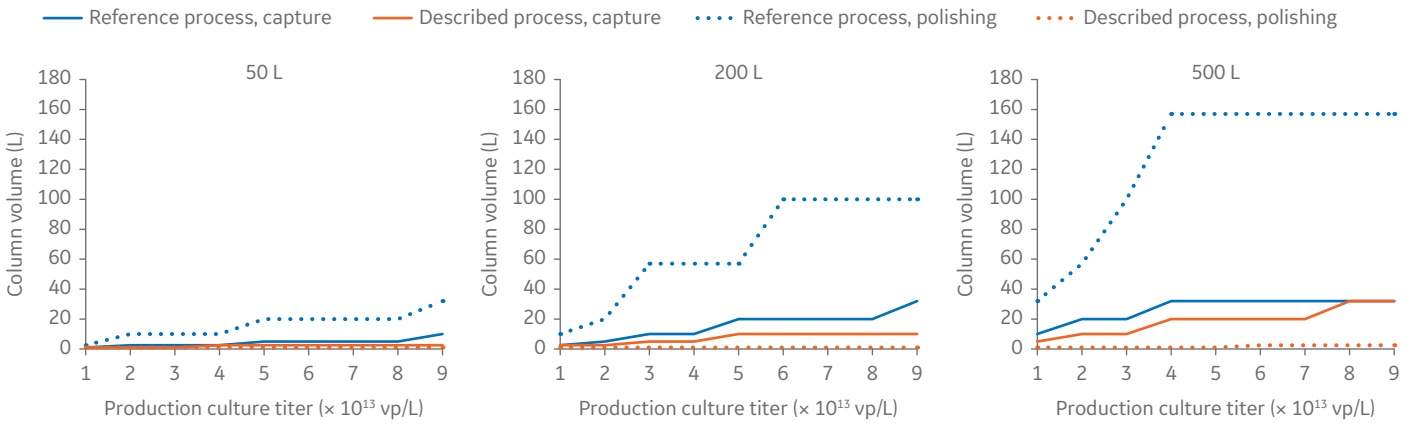

Fig 2. Column sizes for the two processes across different scales and titers in the single-use configuration. A resin with lower capacity, such as Q Sepharose XL, will require a larger column size and more cycles compared with a resin with higher capacity, such as Capto Q ImpRes, for processing the same sample amount. Consequently, even though Q Sepharose XL was assumed to collect a smaller fraction of the column volume in each cycle during elution, the actual elution pool volume will be higher than for Capto Q ImpRes.

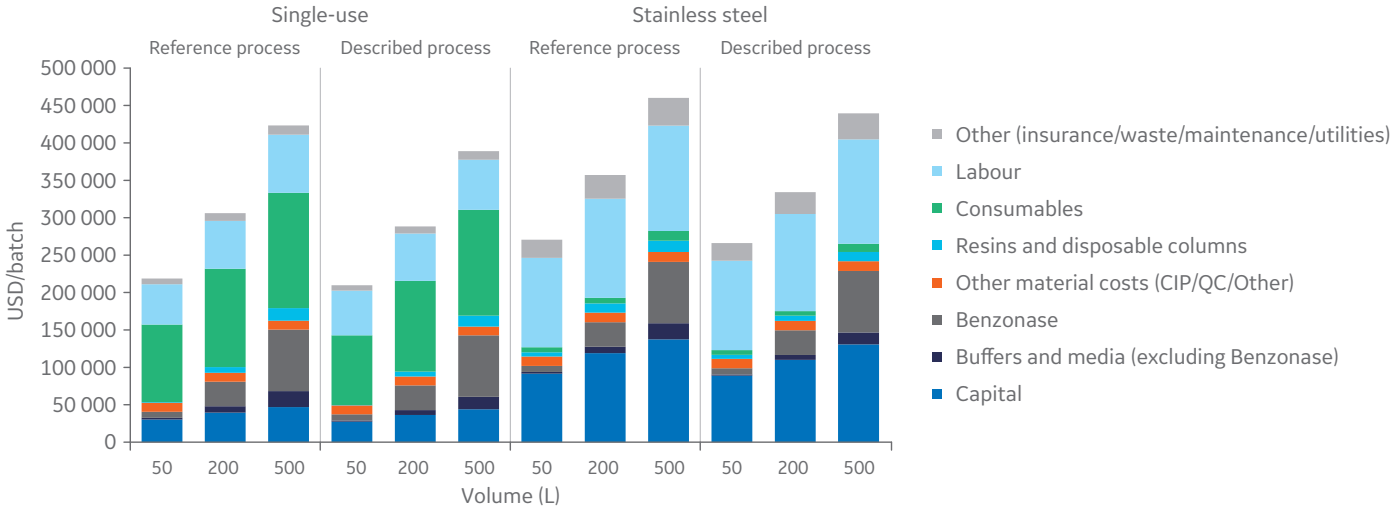

Fig 3. Batch cost breakdown by cost category for the novel and reference processes in both stainless steel and single-use configurations in the single-product scenario. The results are shown for a mid-range titer (5 × 10^{13} vp/L) across all investigated process scales.

Figures 4 to 6 show cost breakdown at a production culture scale of 200 L for all investigated titers. The cost breakdown shows that a major part of the cost for the process is related to consumables such as single-use flow kits for the chromatography system and consumables related to buffer mixing and handling. The larger column volumes required for higher titers also demand larger buffer volumes for processing of a certain amount of sample. Results from this study confirm that preparation and handling of the large buffer volumes required for the reference process contribute to the larger cost of consumables compared with the developed process.

In the investigated scenarios, the single-use process configurations were shown to be more cost-effective that their stainless steel counterparts. The cost breakdown analysis shows that a large part of the cost difference between single-use and stainless-steel configurations is also related to buffer and medium preparation and handling (e.g., capital cost for tanks, as well as materials and labor related to maintenance and cleaning).

The influence of the capital cost on overall batch cost is a function of the number of batches that are produced each year: as the number of batches per year decrease, the capital cost becomes a more dominant factor for all scenarios, while the operational expenditures (including labor and consumables) remain constant (except for reusable materials such as chromatography resins). As seen from the results, the consumable cost for the single-use configuration is replaced by costs for capital investment, other material, labor, and other cost categories related to the setup in the stainless steel configurations, which is expected. As the number of batches per year decrease, the capital cost becomes a more dominant factor for all scenarios. For the multi-product facility, the cost of resins or prepacked columns becomes a larger cost driver, as the resin/column is not fully utilized before replaced.

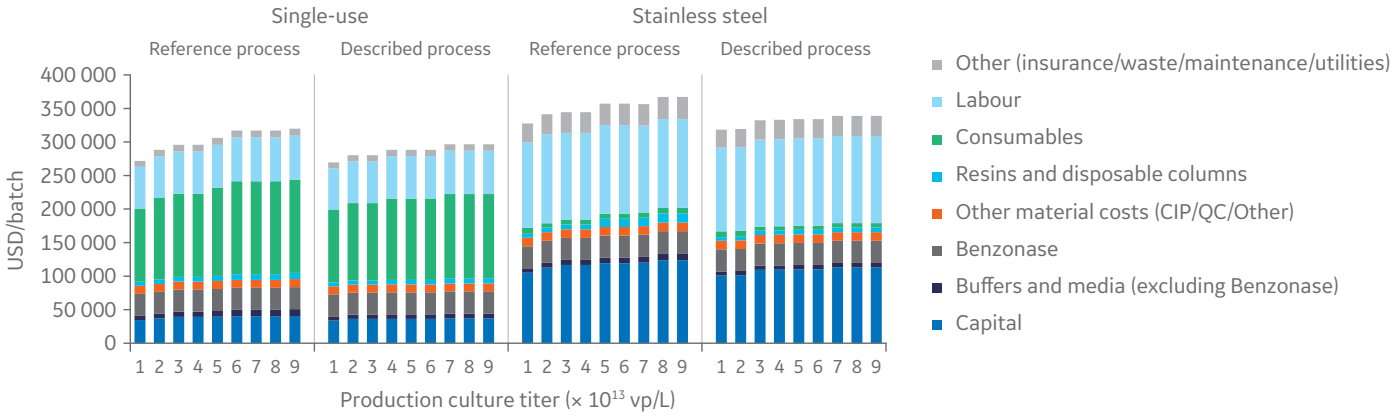

Fig 4. Batch cost breakdown by cost category for the novel and reference processes in both stainless steel and single-use configurations in the single-product scenario. The results are shown for the mid-range process scale (200 L) across all investigated titers.

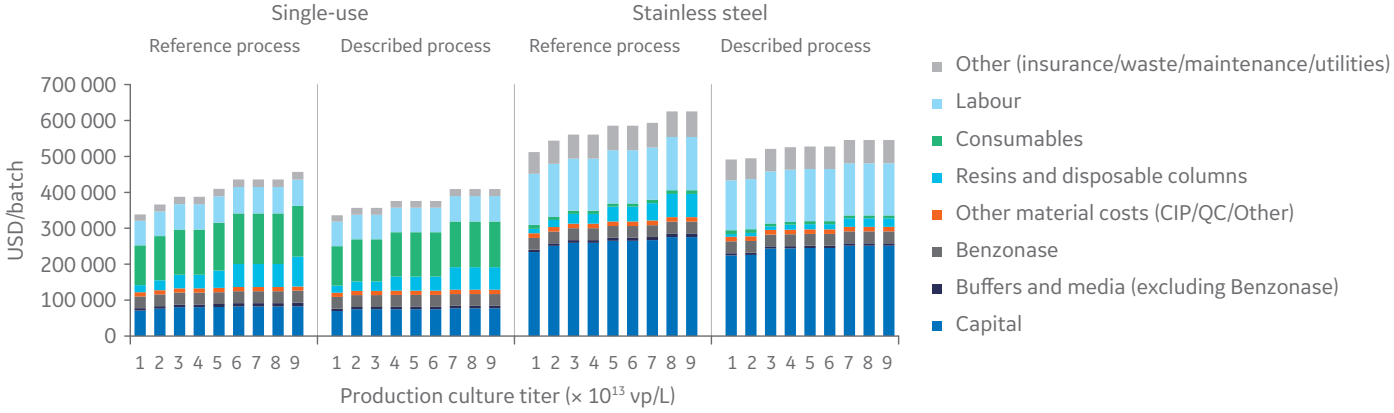

Fig 5. Batch cost breakdown by cost category for the novel and reference processes in both stainless steel and single-use configurations in the multi-product scenario. The results are shown for the mid-range process scale (200 L) across all investigated titers.

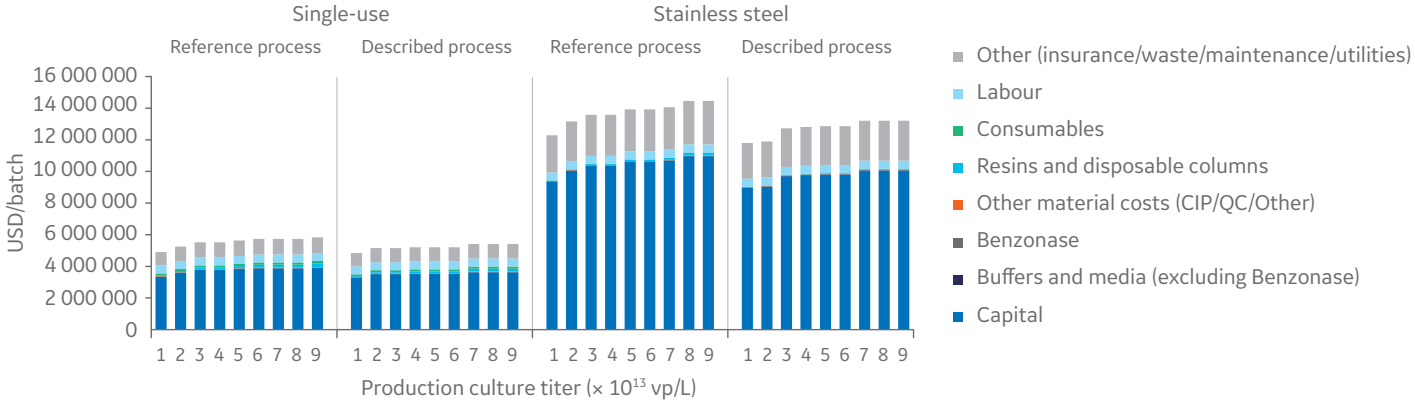

Fig 6. Batch cost breakdown by cost category for the novel and reference processes in both stainless steel and single-use configurations in the single-batch production scenario. The results are shown for the mid-range process scale (200 L) across all investigated titers.

Figures 7 to 9 show process comparisons where a hybrid process configuration has been included, using single-use equipment for buffer and medium preparation and handling and stainless steel equipment for all other process steps. In the single-product scenario, the hybrid configuration performs equal to or better than the single-use process configurations. However, as the number of annual batches decrease, the benefit of a single-use facility is increasing, showing the impact of batch frequency on the overall batch cost and emphasizing the importance of having a liquid handling strategy in place.

Another important aspect when comparing single-use and stainless steel equipment is campaign change-over time and the number of batches possible to produce each year with the different configurations. In the multi-product scenario, with three batches per campaign, about 20% more batches can be produced with the single-use setup. For the single-product scenario, the difference becomes less pronounced. According to the simulations, the single-use processes can be run for 96 to 97 batches in a 12 month campaign, while the stainless-steel processes are limited to 89 batches, representing an approximate 8% difference. Apart from throughput considerations, the annual number of batches possible to produce will also impact the capital contribution to batch cost through the depreciation.

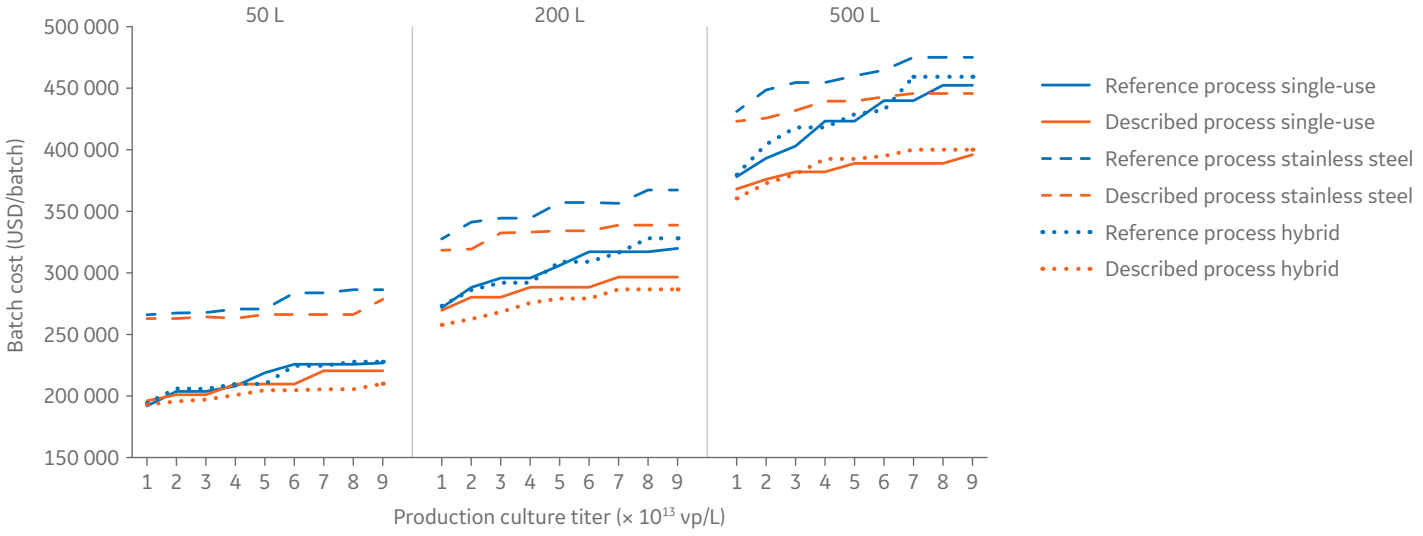

Fig 7. Batch cost comparison across all investigated titers and process scales for the single-product scenario, for stainless steel, single-use and hybrid configurations.

Fig 8. Batch cost comparison across all investigated titers and process scales for the multi-product scenario, for stainless steel, single-use and hybrid configurations.

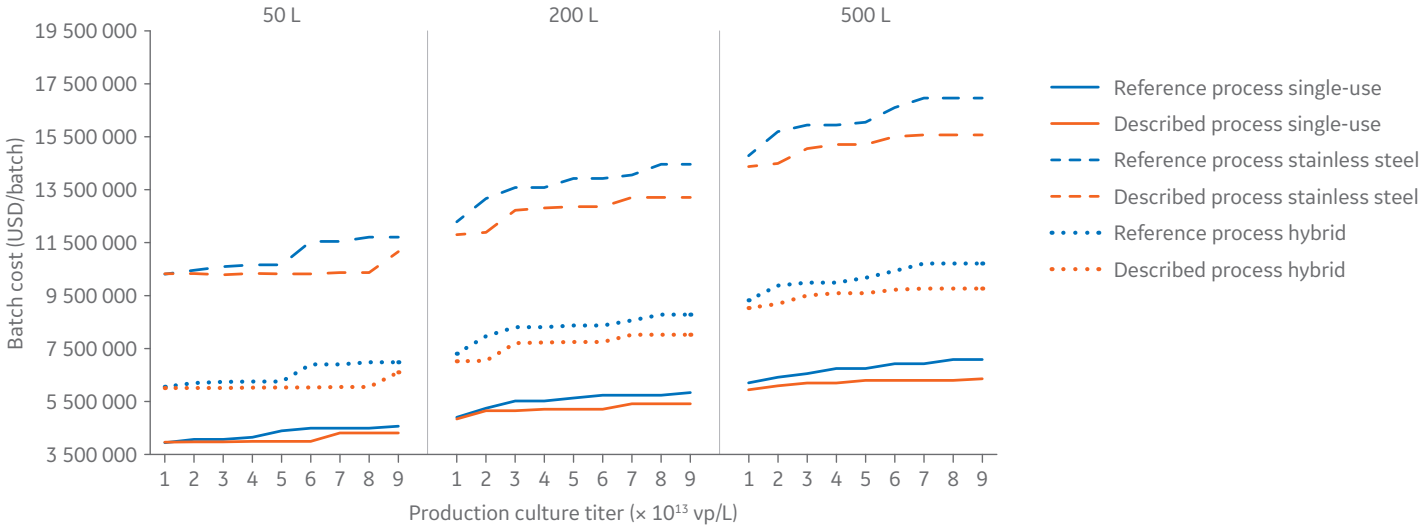

Fig 9. Batch cost comparison across all investigated titers and process scales for the single-batch production scenario, for stainless steel, single-use and hybrid configurations.

In this work, the assumption was made that there is no difference in the lifetime between traditional and prepacked columns. Note that this assumption has not been tested experimentally in this case, and that the actual lifetime of any packed bed (prepacked or otherwise) will vary from process to process. To reduce complexity, the model did also not account for the possibility to repack a failed bed in a traditional column, while a prepacked column must be replaced in the same scenario. It is important to note, however, that column replacement is only relevant in the single-product scenario, being the only scenario where the full lifetime of the resin is utilized.

Conclusion

The main conclusion of this work is that the novel process, based on Capto Q ImpRes capture and Capto Core 700 polishing, is a more cost-efficient alternative than the reference process for industrial production of adenovirus. These findings become increasingly evident at higher process scales, and are due to two main factors: the low elution volume from the capture step and the high capacity of both included resins, keeping the column volumes and number of cycles in both chromatography steps low compared with the reference process.

The modelling output indicates that the single-use configurations have a higher possible annual throughput and lower cost per both batch and dose compared with the stainless steel configurations for the investigated processes and scales. To large part, the lower cost seems to be associated with buffer and medium handling. If this factor is removed (as seen in the hybrid process configuration), the stainless steel configurations have a lower batch cost than corresponding single-use processes for most scales when many consecutive batches are produced without campaign changeover. With fewer batches per campaign, however, the single-use configurations are the most cost-efficient alternatives. This finding shows the importance of having an appropriate liquid handling strategy in place.

Our results show that the novel process is a cost-efficient alternative to the reference process at all investigated scales and scenarios, making it feasible for industrial production of adenovirus, for example, for clinical applications.

Disclaimer

The results and conclusions presented in this theoretical study are valid for this specific study only. Other process conditions and assumptions could have significant impact on the outcome. The model was found to be especially sensitive to titer and yield. Assumptions regarding these, and any other factors, need to be carefully evaluated to ensure a meaningful model output. The figures presented in this study regarding the number of cycles that can be run on packed beds (both traditional and prepacked columns) are theoretical assumptions. The actual number of cycles that can be run on any packed bed will vary from process to process based on a number of factors, such as quality of the feed, efficiency of the CIP, properties of applied liquids, pressure drop over the column, storage conditions, and similar.

References

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