A Software Tool for Assessing the Financial and Technical Impacts of Changing Industrial Bio-Manufacturing Processes

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ABSTRACT:

Growing pressures in the bioprocess industries are driving the need for simulations that rapidly evaluate strategies for achieving improvements in large-scale production. This paper presents a prototype simulation that evaluates the financial and technical impacts of developing and implementing a range of manufacturing changes to a pre-existing industrial process. The simulation evaluates each option with respect to development costs and timescales as well as annual production mass, cost of goods and batch times. These metrics are integrated together using a multi-attribute decision making (MADM) technique to produce a single value that quantitatively evaluates each strategy. The methodology is applied to development strategies being considered for an industrial process operated by Protherics U.K. Limited that manufactures an FDA-approved polyclonal Fab preparation for the treatment of rattlesnake envenomation. In the current process, an ovine serum feed containing anti-venom IgG is subjected to sodium sulphate precipitation to sediment the antibodies. The precipitate is separated from the contaminating supernatant by disk stack centrifugation, after which the IgG molecules are enzymatically digested by papain into their F_{AB} and F_C components. The latter is removed from the process stream by passing it through an ion exchange column, after which the venom-specific Fab is recovered in an affinity step. Process changes considered include replacing the precipitation and centrifugation stages by a synthetic Protein A column step operating in either packed or expanded modes, eliminating the ion exchanger, raising the volume of the ovine feed and increasing the venom-specific IgG titre. Of all the changes examined, using an expanded bed column with the highest increase in IgG titre that could be achieved and the greatest feed volume that could be handled within the facility, combined with the elimination of the ion exchanger results in the best MADM-based value. This would therefore be the most desirable alternative to current operation.

1 INTRODUCTION

1.1 Benefits and challenges of modelling bioprocesses

Dynamic simulations are valuable tools for the bioprocess industry, helping to reduce the costs and timescales involved in developing a process and operating it at large scale [1, 2]. Previously, modelling has been used to assess the merits of different process options for the production of clinical trial material [3] and the full-scale manufacture of therapeutic products [4]. Modelling has also been used to explore interactions between process steps such as between fermentation and initial downstream purification steps for

intracellular proteins [5, 6, 7]. To date, models have focused on generic flowsheets and evaluating them with respect to manufacturing performance metrics such as annual production levels, expenditures and processing times. In contrast, little attention has been paid to simulating the development and implementation of changes which are designed to achieve manufacturing improvements in existing industrial processes. Such changes could include increasing feed volumes, consolidating multiple operations into a single step or removing process steps altogether, with each change having an associated development cost and duration.

1.2 Evaluation of process change strategies

Evaluating the effects of changing a process can be difficult if some performance metrics improve whilst others deteriorate. For example, increasing the feed volume will result in a higher product mass, but may come at the expense of longer processing times. Similarly, removing a process step may reduce manufacturing costs and times, but increase the purification burden downstream and so necessitate revalidation. These conflicts complicate the decision as to whether or not to pursue a new manufacturing strategy. This paper describes a method which simplifies the decision-making process by combining manufacturing and development metrics into a single value that quantifies the desirability of a process change strategy. The utility of the method is illustrated in an industrial case study, where alternative manufacturing strategies are evaluated and ranked in order to identify the most suitable process change option.

1.3 Industrially relevant process change options

1.3.1 Introduction

The following section describes a series of development strategies with potential for achieving manufacturing improvement and are illustrated in more detail in the industrial case study

1.3.2 Increasing feed titres and batch volumes

A common strategy employed to improve production levels is to increase feed titres, such as by the metabolic engineering of microbes used in fermentation [8] or optimisation of immunisation protocols and/or the selective breeding of animals used to provide feedstocks to polyclonal processes. Raising the feed volume to a process will also improve production levels, but without any extra holding vessels or equipment, bottlenecks may arise in production and lead to longer manufacturing times.

1.3.3 Replacing multiple steps with a single operation

Consolidating multiple steps into a single operation has the potential to reduce process costs and times and improve product yield. A strategy that attracts industrial interest is the replacement of potentially time consuming operations such as precipitation or centrifugation by a product-specific affinity chromatographic step [9], operating in either packed or expanded bed modes [10, 11].

1.3.4 Removing a downstream operation

Removing a step may increase product yields and reduce costs and times by virtue of having a shorter process train, but may place a heavier purification duty on steps further on in the flowsheet. This may necessitate altering those downstream steps in order to cope with the greater burden and so require re-validation of those steps.

1.4 Paper structure

This paper is structured as follows: initially, details of the construction of the manufacturing model are provided, followed by an overview of the calculation of development costs and times. The technique which combines multiple performance metrics into a single value is described and then illustrated in an industrial case study.

2 METHODOLOGY

2.1 Modelling the manufacturing process train

The manufacturing model in this research was constructed in Extend™ (version 6, Imagine That, San Jose, California, U.S.A.) [12, 13]. Extend™ contains many 'blocks,' which encapsulate functions such as those needed to manipulate material balance data, those which represent the durations of process steps, those which symbolise resource pools for equipment, vessels, buffers etc. as well as methods to access these pools. Blocks are represented graphically as icons and unit operation sub-models are constructed by dropping suitable combinations of these icons onto the Extend™ workspace and linking them together. Unit operation sub-models are then connected together to produce the complete manufacturing simulation.

2.2 Modelling the costs and times of developing process change options

The development model was established in a spreadsheet. For strategies which involve capital expenditure, costs were calculated by using correlations relating equipment capacity to purchase costs [14], updated to current day prices by assuming a 3% annual inflation. Bioprocess Lang factors [15] were used to calculate other costs such as for installation, instrumentation, validation etc. (**Table 1**). Where a unit operation was eliminated from the flowsheet, the costs of revalidating the process downstream of that point were included. For strategies where the feed titre was improved, the costs of purchasing assay systems which determine titres were included. All developmental costs were treated as exceptional expenditures in the first year of manufacturing after implementing the changes. Durations of the different development strategies were specific to the industrial case study and are discussed later.

Table 1: Lang factors used to calculate development costs (adapted from [15])

	Item	Lang factor (f_i)
1	Capital investment e.g. for columns and filter housings used in case study (λ)	1
2	Qualification and validation	1.06
3	Installation	0.9
4	Instrumentation	0.6
5	Process control	0.37
6	Electrical supply	0.24
7	Detail engineering	0.77
8	Contingency factor	1.15
	Calculated cost	$\lambda \cdot f_8 \cdot \sum_{i=1}^{7} f_i$

2.3 Overall Ranking

For ease of assessing the feasibilities of process change strategies and in order to rank them, a weighted sum additive weighting multi-attribute decision making (MADM) technique was used to combine several output performance metrics into a single value [16].

Initially, results for metrics such as production levels or processing times calculated by manufacturing simulations were normalised to a zero to one scale. The zero bound represents the worst possible value and the unity bound represents the best possible value e.g. for product mass, the normalised value is given in equation (1):

$$N = \frac{M_{actual} - M_{zero-bound}}{M_{unity-bound} - M_{zero-bound}} \quad (1)$$

N represents the normalised product mass, M_{actual} is the actual product mass calculated by a given simulation run, $M_{zero\text{-}bound}$ is the lowest product mass out of all simulation runs and is set to represent the zero bound and $M_{unity\text{-}bound}$ is the highest product mass out of all simulation runs and is set to represent the unity bound. Similar formulae are applied to all other metrics. Every normalised value is then multiplied further by a zero to one weighting. The action of weighting ensures that particular emphasis is placed upon those metrics deemed especially important in the analysis, with the more important metrics attracting higher weightings. The sum of all weighting values again equals one. Applying this approach ensures that the assessment of strategies is consistent with commercial aims and objectives. Addition of component weighted and normalised values for a given strategy as calculated by Equation 1 creates a single metric, the Overall Rank (OR):

$$OR = \sum_{i=1}^{5} w_i N_i \quad (2)$$

w_i and N_i in Equation (2) respectively represent the weighting and the normalised values of the i^{th} performance metric. The Overall Rank again has a value between zero and one, respectively representing the least and most attractive outcomes from developing and implementing process change strategies. The Overall Rank for the existing manufacturing process is also calculated in order to create a benchmark against which proposed process change options can be compared.

2.4 Industrial case study

The method described in section 2.3 was applied to a large-scale process operated by Protherics U.K. Limited (Blaenwaun, Ffostrasol, Llandysul, Wales, U.K.), which manufactures polyclonal F_{AB} (CroFab™) for the treatment of rattlesnake envenomation [17, 18, 19]. A variety of manufacturing alternatives are available which offer the potential to boost annual product mass whilst reducing the costs and times involved in processing. Manufacturing metrics (product mass, cost per gram and batch time) and the duration and expenditure of developing process changes were combined into an Overall Rank to determine the best alternative to adopt. In the current process (**Figure 1**), sodium sulphate precipitation and disk stack centrifugation are used to purify and concentrate an ovine serum feed containing anti-venom IgG, prior to its proteolytic digestion by papain to generate F_{AB} and F_C fragments. The F_C portion is removed by an ion exchanger before F_{AB}-specific affinity chromatography is applied to yield the anti-venom F_{AB} product. Several clarifying depth filtration steps and concentrating ultrafiltration steps are also employed between these stages. The process is operated for four polyclonal derived F_{AB}s from sera hyperimmunised with four types of rattlesnake venom. The outputs are then blended together, concentrated and filtered to generate the purified product.

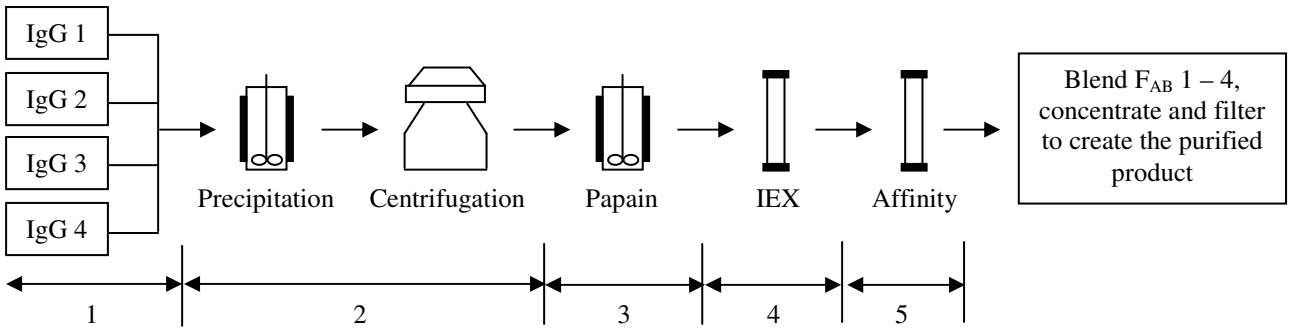


Figure 1: Current process flowsheet, identifying the main unit operations: numbers specify groups of steps to which mass balance yield fractions were assigned in order to calculate the overall product yield. The process is operated separately for four feed sera, each containing one of the mono-specific anti-venom IgGs. Individual affinity purified batches of anti-venom F_{AB} are blended, concentrated and filtered to produce the final product

2.5 Process change strategies

2.5.1 Increase IgG feed titres and batch volumes to the process

Given adequate funding and time, it is feasible to suggest that the venom-specific IgG titre in the ovine feed could be raised by up to 80% by selectively breeding sheep flocks and also optimising the immunisation protocol. It was assumed that serum titres would increase linearly with development time (see **Table 3** later). Although techniques such as ELISA can be used to determine IgG concentrations, their uses are limited by the long analytical times taken when processing large numbers of samples [20]. Alternatives as biosensor assays (Biacore International Aktiebolag P.L.C., Uppsala, Sweden) permit rapid evaluation of specific antibody titres for many serum samples and can therefore be used to screen sheep and identify those which are the highest responders. The resulting Improvements in final product mass resulting from higher feed titres are self evident, but require significant initial capital investment when purchasing the assay units. In parallel to this, a rise in feed volume up to the 1000 L maximum that can be accommodated within the facility was also considered.

2.5.2 Replacement of precipitation and centrifugation by a single column capture step

Chromatographic capture of feed IgG (either in packed or expanded modes) has been proposed using a synthetic protein A resin (MAbsorbent® A2P – ProMetic BioSciences Limited, Cambridge, U.K.), followed by ultrafiltration to concentrate and diafilter the eluate. Comparable IgG purities and recoveries between the MAbsorbent® A2P and sodium sulphate precipitation steps have been achieved [21]. A synthetic ligand is used instead of recombinant Protein A [22], because the latter displays only weak affinity for sheep antibodies [23]. The synthetic material has demonstrated a higher capacity and also provides other advantages, such as a lower cost and an ability to tolerate the harsh cleaning conditions needed when subjected to a crude feedstock such as ovine serum.

2.5.3 Removal of the ion exchange step

Elimination of the ion exchange step from the current flowsheet (**Figure 1**) could potentially reduce processing time and costs, but would necessitate revalidation of the process further downstream. Additionally, loading digested IgG directly onto the affinity column may result in non-specific adsorption of impurities to the F_{AB}-specific affinity matrix and an extra wash step would therefore be needed prior to elution to eliminate these impurities and so ensure that the eluate still met specification. This assumption was based on a similar process operated by Protherics for the purification of another polyclonal F_{AB}

product called DigiFab™ [24], in which digested IgG is loaded directly onto a F_{AB} specific affinity column without prior purification on an ion exchanger and where a wash step is used to eliminate non-specifically adsorbed material such as F_C prior to elution.

2.6 Modelling details of the case study

2.6.1 Introduction

Model construction and execution took place on a 1.4 GHz Pentium M 256 MB RAM computer running Microsoft® Windows XP (Microsoft Corporation, Washington, U.S.A.). Input model data were entered into a Visual Basic for Applications user interface (within Microsoft® Excel XP) and connected to Extend™. The structure of the manufacturing model is outlined in **Figure 2**.

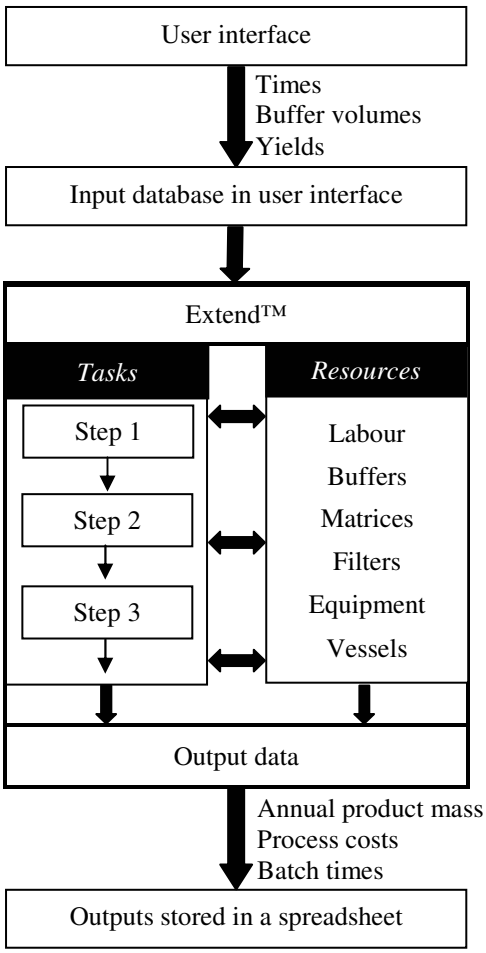


Figure 2: Structure of the manufacturing model

2.6.2 Manufacturing model

Data used to calculate process costs, durations and mass balances were provided by Protherics (**Table 2**). Owing to corporate restrictions, manufacturing costs and detailed processing conditions used for model construction have not been reported in this paper. Venom-specific F_{AB} mass balances were calculated in Extend™ using data provided by Protherics which specified yields achieved by groups of unit operations (**Figure 1**). The manufacturing model accumulates costs for purchasing new batches of resources such as matrices or membranes when previous stocks are exhausted. For the titre improvement option, operational costs for the Biacore system were negligible (~0.1%) relative to purchasing costs and therefore were not included in the analysis.

Table 2: Sample input data

Variable	Value
Assumed current feed volume for each feed F_{AB} stream [L]	600
Number of F_{AB} types processed [-]	4
Assumed number of blended batches manufactured per annum [-]	12
Assumed current initial total IgG titre [g/L]	30
Assumed current initial venom-specific IgG titre [g/L]	8
Overall process duration for the first blended batch in current operation [hrs]	623
Current overall yield per blended batch [%]	26%
Percentage IgG recovery using MAbsorbent A2P [%]	95%

2.6.3 Development model

Table 3 gives the times needed to develop the options and transfer them into the manufacturing facility. Development costs were calculated as detailed previously and where combinations of options were modelled, individual development costs were added together.

Table 3: Assumed durations for developing and implementing process change strategies. Values were estimated following discussions with Protherics

Process change strategy	Estimated duration (months)	Tasks involved
Titre improvement	Up to 19	One month to develop the Biacore assay; six months to write a validation protocol, undertake all the experiments and sign off the assay; two months to undertake trials on small sheep flocks and then scale up the immunisation protocol. Furthermore, based on an assumed 8% increase in titre per month, up to a maximum 80% rise, up to 10 further months would be needed
Packed and expanded bed IgG capture	6	Purchasing, validating and qualifying the column and the subsequent ultrafilter as well as installing the associated instrumentation, process control equipment and electrical supply and then revalidating the remainder of the process downstream of where the centrifuge was originally
Removing the ion exchange step	5	Development of the extra affinity wash step and revalidation of the affinity step and the steps downstream

2.6.4 Modelling assumptions

The following lists some of the key assumptions that were made after discussions with Protherics in order to construct the model:

1) The flowrate in the packed bed model was half of that used for expanded bed operation and expanded bed elution volumes were half of those for the packed bed model
2) Currently, the MAbsorbent® A2P resin is not available on expanded bed adsorption beads, but for the purposes of the analysis, it was assumed that the binding and purification characteristics of the MAbsorbent® A2P matrix were identical in packed and expanded modes (an assumption that would need to be verified experimentally)
3) IgG feed titres for the four feed sera were the same for every batch of starting serum
4) Sufficient space is available in the facility to house any extra equipment required
5) The entire time-course of the development involved for any of the options was assumed to occur before their implementation in the manufacturing facility and no time slippage contingency would need to be allowed for in developing and implementing a given process change option
6) Where combinations of options were modelled, it was assumed that sufficient resources (e.g. staff or funding) would be allocated to allow different projects to start simultaneously, meaning that the total duration for those combinations was set equal to the duration of the slowest strategy to be implemented
7) The number of batches manufactured per annum remains unchanged (**Table 2**)

2.6.5 Application of the multi-attribute-decision-making technique

Table 4 provides the metrics used in the assessment of the industrial development options and values assigned to the bounds for normalisation. Values quoted are the highest and lowest values from the entire set of simulation results for all options and combinations of options examined. Development times were normalised relative to the strategy taking the longest time i.e. increasing the IgG titre (maximum 19 months – rank of 0; see **Table 4**). The current process with no requirement for development was assigned a

normalised rank of 1 for each of the two development metrics. Overall Ranks for each process change option were calculated by equations 1 and 2.

Table 4: Values used to normalise the output values of the five performance metrics onto a zero to one scale. The highest and lowest values from the entire set of simulation results were used to set the zero and unity bound values for the five metrics. Manufacturing batch time was that measured for the first blended batch

	Metric	Zero bound	Unity bound
Manufacturing	Annual product mass (g F_{AB})	~40,000	~ 147,000
	Cost per gram (£/g F_{AB})	Current level	70% lower
	Batch time (hours)	~ 790	~ 470
Development	Cost (£)	Maximum value from all model runs	0
	Time (months)	19 months	0 months

Based on discussions with Protherics, the annual manufacturing cost per gram and F_{AB} product mass were taken to be the most important metrics and assigned weights of 1/4 each, whilst the other three were equally weighting (1/6), giving a sum of 1. The Overall Rank benchmark for the current process was calculated to be 0.42 using the current manufacturing conditions given in **Table 2**.

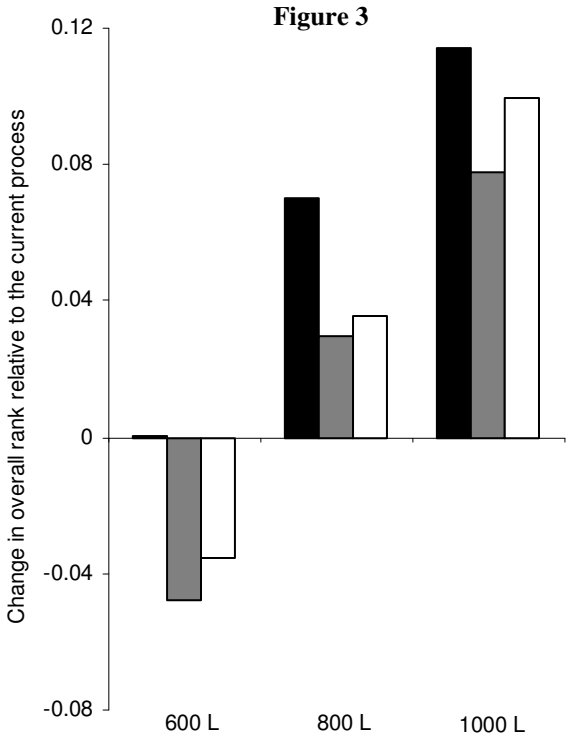
3 RESULTS AND DISCUSSION

3.1 Simulation results

The graphs in this section plot the change in Overall Rank relative to that of the current operation (i.e. the existing process flowsheet operated with the current feed volume and IgG titre – see Table 2). Outcomes of simulated options that are superior to the current operation appear above the x axis or the x–y plane, for 2 and 3D graphs respectively, whilst inferior options appear below.

3.2 Impact of increasing the feed volume

Figure 3 shows the impact of increasing the feed volume to the current manufacturing process and to variants based on the use of MAbsorbent® A2P. Use of either packed or expanded bed column capture of IgG from a 600 L feed results in an inferior solution relative to the current manufacturing process. A breakdown of the normalised and weighted individual performance metrics in Figure 4 indicates that although the manufacturing ranks for the MAbsorbent® A2P column-based options exceed those for the current process, there is a heavy price to be paid in terms of additional development costs. The methodology used in this paper calculates development costs as exceptional expenditures in year one and balances these against the manufacturing metrics shown in Table 4 for a single year's worth of production. Calculated in this way, the expenditure in development that has to be borne more than outweighs the advantages of increased annual product mass, reduced cost per gram and decreased batch times. In addition, Figure 3 indicates that when operating with a 600 L feed, the Overall Rank of the packed bed option is inferior to the expanded bed option, with Figure 4 showing that operation in expanded mode reduces the cost per gram and batch time to a greater extent than if using a packed bed.



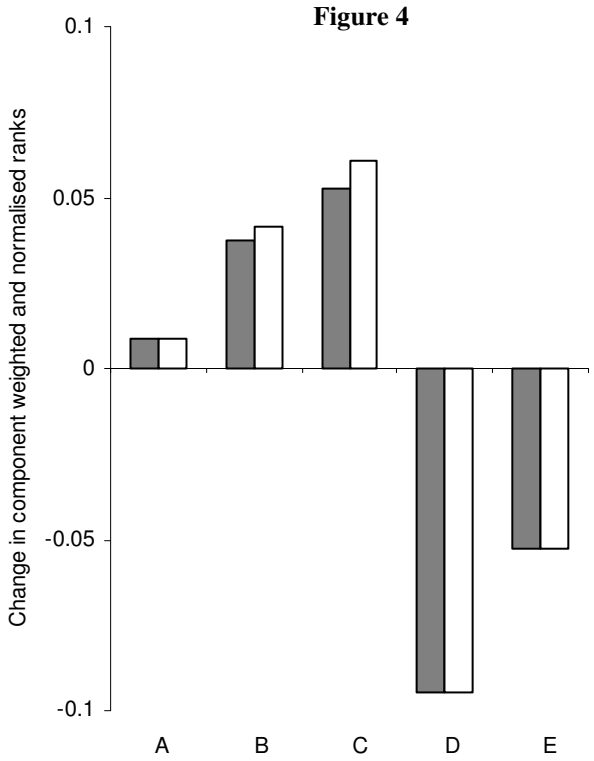


Figure 3: Impact of operating with 600 L, 800 L and 1000 feed volumes on Overall Rank for the current (black bars), packed (grey bars) and expanded bed (white bars) processes

Figure 4: Breakdown of Figure 3 of the individual ranks for the five metrics for the packed bed (grey bars) and expanded bed (white bars) processes operating with a 600 L feed. Values plotted for each metric show the change relative to the weighted and normalised value for that metric in the current process flowsheet operated with a 600 L feed. A = manufacturing F_{AB} mass; B = manufacturing cost per gram; C = manufacturing batch time; D = development cost; E = development time

As the process volume is increased to 800 L and then 1000 L, the Overall Rank increases for all three options by virtue of increased annual production and reduced manufacturing cost per gram values. Nonetheless, the current process flowsheet still outperforms the other two options at 800 L and 1000 L scales of operation. This conclusion again arises from treating the development costs as exceptional expenditures. Calculated in this way, there appears to be no justification for changing from the current manufacturing process to either IgG column capture method on their own, even if combined with an increased feed volume to 1000 L. If, on the other hand, the development cost was depreciated over several years of manufacturing operations, it is likely that the improved manufacturing performance in that timeframe would merit the developmental expenditure.

3.3 Impact of removing the ion exchanger

Figure 5 demonstrates the impact of removing the ion exchange step at 600 L, 800 L and 1000 L feed volumes from the current process and the packed and expanded bed variants. With a 600 L feed, a small reduction in rank is seen for the current process, because improvements achieved in the manufacturing metrics are just offset by development costs and durations. This suggests that removing the ion exchange step on its own from the current process would not be favourable. Increasing the feed volume to 800 L and 1000 L and eliminating the ion exchanger is beneficial, but still inferior to the option of simply employing the existing process with 800 L and 1000 L feed volumes. Conversely, removing the ion exchange step from the packed and expanded bed-based

variants represents a favourable process change. This is due to cumulative improvements in the manufacturing metrics which significantly outweigh heavy development costs. The implication is that using direct column capture instead of precipitation/centrifugation whilst also eliminating the ion exchange step can achieve a superior process compared to current operation.

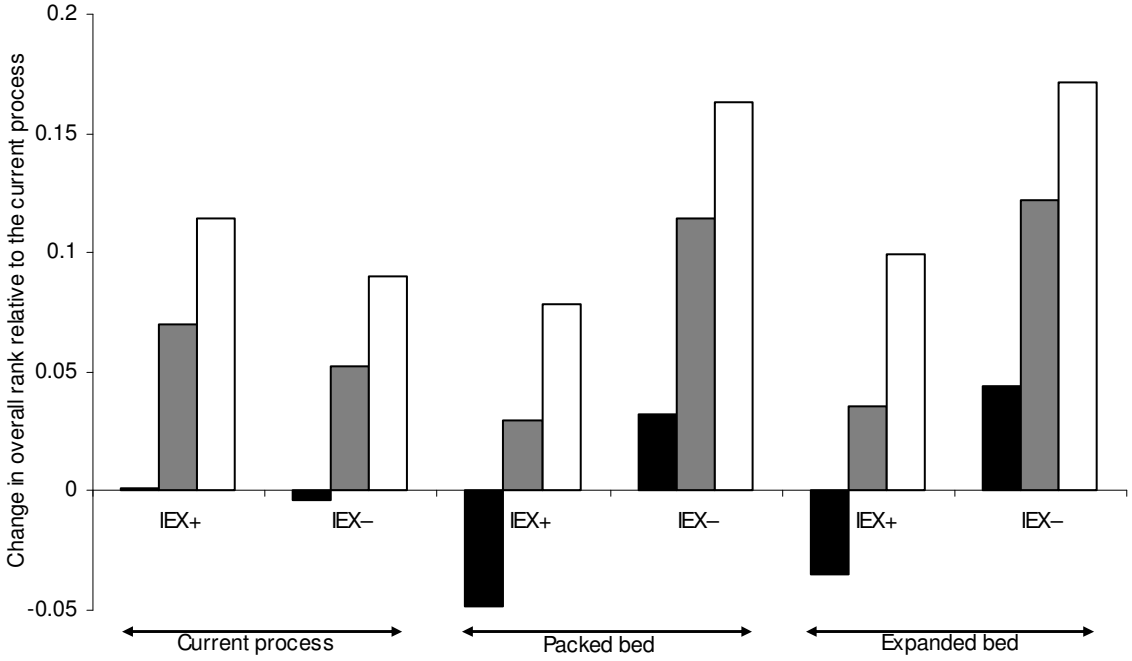


Figure 5: Impact of retaining and removing the ion exchange step from the current, packed and expanded bed processes on Overall Rank, operating with feed volumes of 600 L (black bars), 800 L (grey bars) and 1000 L (white bars). Presence or absence of the ion exchanger step is denoted by IEX+ and IEX- respectively

3.4 Impact of removing the ion exchanger and also increasing the IgG titre

Figure 6 shows the effects of increasing the titre by up to 80% and also removing the ion exchange step upon Overall Rank at 600 L, 800 L and 1000 L feed volumes for the current process and the expanded bed option. The expanded bed option outperforms the packed bed option, which in turn outperforms the current flowsheet for all combinations of feed volumes and titres tested. In order to achieve superior operation with a 600 L feed than at present when operating without the ion exchange step, a titre increase of 20% is needed

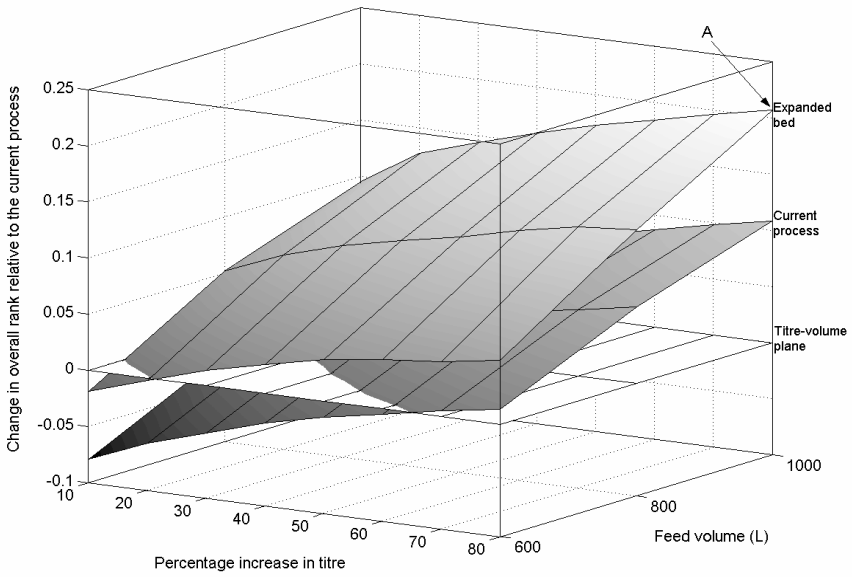


Figure 6: Impact of removing the ion exchange step and increasing the venom-specific IgG feed titre on Overall Rank for the current and expanded bed flowsheets, operating with 600 L, 800 L and 1000 L feed volumes. Superior performance relative to current operation is achieved by combinations of feed volume and titre above the titre-volume plane

for the expanded bed option, whilst for the current process flowsheet, an increase of 65% is required. At 800 L, a titre improvement of 20% is required for the current process without the ion exchange step in order to achieve a superior Overall Rank, whilst operating with 800 L for the packed and expanded bed options without the ion exchanger results in a better alternative to the current operation over the complete 80% titre range. In all three cases at 1000 L, the altered process flowsheets are superior to the current operation. In particular, the Overall Rank for the expanded bed option without the ion exchanger operating with a 1000 L feed (66% higher than at present) and an 80% higher IgG titre was the largest seen out of all the options examined (Point A on Figure 8). This provides the greatest return in manufacturing for the investment in development and hence would be the most desirable replacement to the current operation.

4 CONCLUSIONS

This research utilised a software tool to assess the impact of developing and implementing manufacturing alternatives to an FDA-approved production–scale process, subject to predicted changes in input variables such as feed volume or product titre. Each option was assessed using a multi-attribute decision making technique, both in terms of manufactured product mass, costs of goods and batch times as well as developmental costs and timescales. In the existing process, an IgG feed is subjected to precipitation and centrifugation, followed by papain digestion which cleaves the antibody molecules into F_{AB} and F_C fragments. The F_C fragments are eliminated by an ion exchange step, after which affinity chromatography yields the F_{AB} product. Of all the process changes evaluated, combining a modelled expanded bed column with a 66% higher feed volume and an 80% higher titre together with the elimination of the ion exchange step delivered the most attractive alternative to current operation. Such data can be used for the rapid assessment of process alternatives in line with commercial aims and objectives and so ensure that the most attractive production scenario is chosen to replace current operation.

ABBREVIATIONS AND SYMBOLS

M_A: Actual product mass (g F_{AB})
M_0: Lowest product mass set to represent the zero bound in normalisation (g F_{AB})
M_1: Highest product mass set to represent the unity bound in normalisation (g F_{AB})
N_i: Normalised value of the i^{th} performance metric (-)
OR: Overall Rank (-)
w_i: Weighting of the ith performance metric (-)

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