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Review

Process economics of industrial monoclonal antibody manufacture☆

Suzanne S. Farid

The Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, UK

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Abstract

Pressures for cost-effective manufacture of antibodies are growing given their high doses and increasing market potential that have resulted in significant increases in total site capacities of up to 200,000 L. This paper focuses on the process economic issues associated with manufacturing antibodies and reviews the cost studies published in the literature; many of the issues highlighted are not only specific to antibodies but also apply to recombinant proteins. Data collated at UCL suggest current benchmark investment costs of \$660–\$1580/ft^2 (\$7130–\$17,000/m^2) and \$1765–\$4220/L for antibody manufacturing facilities with total site capacities in the range of 20,000–200,000 L; the limitations of the data are highlighted. The complications with deriving benchmark cost of goods per gram (COG/g) values are discussed, stressing the importance of stating the annual production rate and either titre or fermentation capacity with the cost so as to allow comparisons. The uses and limitations of the methods for cost analysis and the available software tools for process economics are presented. Specific examples found in the literature of process economic studies related to antibody manufacture for different expression systems are reviewed. The key economic drivers are identified; factors such as fermentation titre and overall yield are critical determinants of economic success. Future trends in antibody manufacture that are driven by economic pressures are discussed, such as the use of alternative expression systems (e.g. transgenics, *E. coli* and yeast), disposables, and improvements to downstream technology. The hidden costs and the challenges in each case are highlighted.

Keywords: Process economics; Cost of goods (COG); Benchmark; Monoclonal antibody; Production/manufacture; Mammalian cell culture; Transgenic; *E. coli*

Contents

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E-mail address: s.farid@ucl.ac.uk.

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1. Introduction

Monoclonal antibodies have played a role in several of the important advances in pharmacotherapy; agents such as Synagis™, Herceptin™ and Remicade™ have contributed to the treatment of infectious diseases, cancer and autoimmune diseases, respectively [1]. However, they are amongst the most expensive of all drugs where the annual cost per patient can reach $35,000 for antibodies treating cancer conditions. These high prices are a reflection of the fact that antibodies are now marketed for chronic conditions and of their relatively low potency which results in the need for high cumulative doses (grams rather than milligrams). Consequently expensive large-scale production capacity is required to fulfil market demand and produce 10–100 s kg/year [2–4]. The importance of access to large scale manufacturing capacity was particularly noticed when demand for the antibody fusion protein, Enbrel™, exceeded available capacity in 2000. These trends, as well as industry pressures, have triggered renewed interest in assessing the financial burden associated with manufacturing and hence its contribution to overall corporate economic success.

Most of the approved monoclonal antibodies are manufactured using similar processes [3–5]; the majority use batch/fed-batch culture using mammalian cells followed by purification steps that rely primarily on chromatography with intermediate filtration and viral clearance operations. Pressures to drive down manufacturing costs have also encouraged the search for alternative production technologies, such as the use of transgenic expression systems or *E. coli* and yeast for antibody fragments. With increasing titres in mammalian cell cultures, there are also pressures to improve downstream technology. For antibodies to reach their full commercial potential, all improvement efforts need to demonstrate that they can bring down the cost of antibodies. This paper analyses the process economic issues associated with manufacturing antibodies and reviews the cost studies published in the literature; many of the issues highlighted are not only specific to antibodies but also apply to recombinant proteins.

Section 2 provides benchmark data on capital investment and cost of goods (COG) in antibody manufacturing facilities. The methods for cost analysis and the current status of software tools for process economics are discussed in Sections 3 and 4. Specific examples found in the literature of process economic studies related to antibody manufacture are discussed in Section 5. Finally, Section 6 speculates on the economic challenges with likely routes of future antibody production.

2. Benchmark manufacturing costs

2.1. Benchmark capital investment costs for commercial antibody production facilities

The fixed capital investment is often defined as the capital paid to the contractors to build the plant ready for start-up. It includes the cost of the buildings complete with all the equipment, piping, instrumentation and utilities installed; in addition indirect costs such as the design and engineering costs as well as the contractor's fees need to be accounted for. Investment costs for commercial antibody production facilities are reported to range from $40 M to $650 M. Benchmarking capital investment costs is complicated by the fact that an indication of the facility size, i.e. floor area or bioreactor capacity, is not always stated with the cost; but these are useful indicators of the scale of the facility to benchmark against.

Recently there has been significant activity in establishing antibody manufacturing facilities and new capacity is still being built to produce hundreds of kilograms of antibodies per year; this can be attributed to the low potency and hence high doses of antibodies [6]. Estimates of recent investment costs have been collected at UCL for antibody facilities using mammalian cell culture. A summary of these costs where an indication of the facility size is specified is provided in Table 1. The table indicates costs reported for facilities recently built on new sites or as expansions to current sites by companies such as Amgen, Biogen, Boehringer Ingelheim, Genentech, Imclone and Lonza. Most are multiproduct facilities used to produce marketed antibodies such as Rituxan™, Herceptin™, Avastin™, Xolair™, Synagis™, Erbitux™ and Antegren™ and the antibody-based fusion proteins Enbrel™ and Amevive™. Hence, these multiproduct facilities now reach sizes of 500,000 ft^2 (46,450 m^2) and total bioreactor capacities of up to 200,000 L, typically achieved with multiple bioreactors of up to 25,000 L. From the reported costs in Table 1, it is possible to derive current benchmark investment costs relative to facility size of $660–$1580/ft^2 ($7130–$17,000/m^2). These ranges are similar to estimates in the literature [7–9] of generalised benchmark construction costs for a GMP biopharmaceutical facility that lie in the range $600–$1500/ft^2. Pavlotsky [9] also found that non-USA-based facilities cost approximately 28% less than US-based facilities. Rogers [10] provide a useful breakdown of facility and process-related construction costs for different areas such as fermentation/purification, finishing areas, utilities and offices. For bioprocess engineers it can also be useful to

Table 1
Capital investment costs for antibody facilities using mammalian cell culture

Manufacturing facility	Date facility completed	Capital investment (US $M)	Area (sq ft)	Production bioreactor capacity		
				Number	Size (L)	Total (L)
Genentech—Vacaville, CA, USA	2000	250	310000	8	12000	96000
Imclone—Branchburg BB36, NJ, USA	2001	53	80000	3	10000	30000
Biogen—LSM, RTP, NC, USA	2001	175	245000	6	15000	90000
Boehringer ingelheim expansion—Biberach, Germany	2003	315	–	6	15000	90000
Lonza biologics expansion—Portsmouth, NH, USA	2004	207	270000	3	20000	60000
Amgen—BioNext, West Greenwich, RI, USA	2005	500	500000	9	20000	180000
Genentech NIMO**—Oceanside, CA, USA	2005	380	470000	6	15000	90000
Imclone—Branchburg BB50, NJ, USA	2005	260	250000	9	11000	99000
Biogen Idec—Hillerød, Denmark	2007*	350	366000	6	15000	90000
Lonza biologics—Tuas, Singapore	2009*	250	–	4	20000	80000
Genentech expansion—Vacaville, CA, USA	2009*	600	380000	8	25000	200000

*Expected completion date. **Originally built by Biogen Idec and sold to Genentech in 2005.

have benchmark investment costs relative to the total bioreactor capacity at production scale since this is often estimated based on forecasted demand, titre, yield and number of batches per year. The data collected in Table 1 suggest benchmark investment costs relative to bioreactor capacity of $1765–$4220/L for capacities in the range of 20,000–200,000 L; hence average investment costs for facilities with a capacity of 20,000 L and 200,000 L are $60 M and $600 M, respectively. It has been suggested that validation accounts for 10–20% of the cost of the plant [11,12]. Of course these benchmark costs represent order-of-magnitude estimates useful for preliminary budget cost estimates; better cost estimates can be derived from more detailed modelling and with knowledge of factors such as the number of purification trains. The data collected in Table 1 has certain limitations which may contribute to the wide ranges in the benchmark costs. For example, it is not always clear whether the costs and floor areas account for warehouses, support facilities and office areas or just the bulk manufacturing facility or whether indirect costs are included.

The challenges and pressures to reduce costs have encouraged the search for alternative production technologies such as the use of *E. coli*, yeast and transgenics. Increasing interest can be attributed to claims of a lower cost of goods and, in the case of transgenics, increased flexibility to modulate capacity, when compared with mammalian cell culture. Onigman [13] provided projected estimates of the investment required for the production of antibodies using mammalian cell culture and transgenic goat's milk. The investment for the latter included the cost of herd scale-up, farm and dairy facilities and purification facilities. A summary of these costs for high dose products is shown in Table 2. These predictions suggest that at such large scales, the capital requirements for transgenic-based processes could be up to half those for mammalian-based processes. Hence, Onigman [13] suggest that transgenics are a viable alternative to cell culture, especially for scaling up production to the ton-scale. However, these calculations were based on a 10-fold increase in titre in goat's milk relative to cell culture from 0.3–0.6 g/L to 5 g/L. Given that more recently cell culture titres of 5 g/L have been reported in the literature [2,6,14], it would be useful to see further studies examining whether such benefits with transgenics will still hold. It has been suggested that improved titres in mammalian cell cultures are dampening the prospects for transgenics [15].

2.2. Benchmark cost of goods for commercial antibody production facilities

Manufacturing cost of goods values are reported to represent up to 20–25% of sales [16,17]. Rosenberg [18] indicated that process development and clinical manufacturing costs could represent 40–60% of development costs; the author also suggested that these costs could equal or exceed clinical trial costs. Significant pressures exist to increase production scales for antibodies that are used at high doses (>1 g per patient per year) and have large potential markets (>500,000 patients) [19]. This has triggered a drive to reduce manufacturing costs at the commercial scale by an order of magnitude from $1000's per gram to $100's per gram [20] or even $10's per gram [21].

In reviewing literature sources a fair comparison of costs per gram is further complicated by the fact that the annual production rate (kg/year) and either titre (g/L) or fermentation capacity (L) are not always stated with the cost; but the lower the production rate and titre and the smaller the scale, the higher the cost per gram. In addition the basis for the cost calculations is not always specified making reported values difficult to interpret and to compare. Werner [22] provides a useful illustration of the impact of titre on the number of 10,000 L bioreactors required and hence the COG/g to produce 250 kg/year (Table 3). This

Table 2
Comparison of capital investment estimates for high dose antibody production using different expression technologies[a]

Expression system	Capital investment		
	250 kg/year	500 kg/year	1000 kg/year
Mammalian cell culture	$125–$145 M	$150–$205 M	$220–$295 M
Transgenic goats	$30–$75 M	$75–$90 M	$80–105 M

[a] Adapted from Onigman [13]. For mammalian cell culture a titre of 300–600 mg/L and 18 batches were assumed. For the transgenic goats a titre of 5 g/L of milk was assumed.

Table 3
COG/g for mammalian systems producing antibodies for different titres[a]

Annual production rate (kg/year)	Titre (g/L)	Production bioreactor capacity (L)	COG/g ($/g)	COG/year ($M/year)
250	1	20000	260	65
250	0.1	310000	1500	375

[a] Source: Werner [22].

Table 4
Comparison of cost per gram estimates for given production rates

Expression system	Cost of goods per gram ($/g)	
	100 kg/year	1000 kg/year
CHO cells	300–3000[a]	–
Transgenic goat	105[a]	36[a]
Transgenic corn	50[b]	14[b]

[a] Source: Young et al. [64].
[b] Source: Mison and Curling [66].

resulted in the COG lowering from US$1600/g to US$ 260/g, reflecting a reduction in the annual cost of goods from US$ 375 M to US$ 65 M. These figures can act as useful benchmark COG values. However, more data is needed to also see how COG/g values change with production rate (kg/year). According to Myers [23], costs for monoclonal antibody production are split with one-third attributable to cell culture, one-third to purification and one-third to support. However, one would expect this ratio to change with scale. Further issues that complicate cost comparisons are whether the COG values quoted are for bulk manufacture of the drug substance or include the formulation steps and whether they are for single-product dedicated facilities or for multi-product facilities.

Current estimates of the cost per gram for different expression technologies where the production rate is specified are summarised in Table 4. These results suggest that production in transgenic corn is the cheapest, followed by transgenic goat and then mammalian cell culture. In particular, the transgenic-based systems appear to offer a 1–2 order of magnitude reduction in the cost of goods per gram at the 100 kg/year scale. More detailed costs studies related to transgenics are described in Section 5. However, a more thorough assessment of the economics of different expression technologies probably needs to be addressed in order to provide a fair comparison to fermentation and transgenic-based processes; hidden costs need to be accounted for such as the risks of contamination, the ethical issues that may affect market penetration and the impact of validation issues which have yet to be resolved for transgenic sources.

3. Models to predict costs

3.1. Predicting capital investment

Process engineers often employ a factorial method for capital cost estimation. Factorial estimates are based on the analysis of costs of previous projects and relate the total capital cost of the plant to the cost of the equipment in the plant. The factorial method is often attributed to Lang [24] where the fixed capital investment is estimated by multiplying the total equipment purchase cost by a factor, usually termed the "Lang factor". For chemical engineering facilities, the value of the factor typically lies in the range of 3–5 [25,26]. Comprehensive studies to determine an appropriate range of Lang factors for biopharmaceutical facilities are not presented in the literature. However, values in the range of 3.3–8.1 have been suggested for biopharmaceutical facilities [9,11,27–29].

A key stage in determining the capital required for a production facility is ascertaining the total equipment purchase cost. To estimate the cost of a new piece of equipment, process engineers often use the well-known six-tenths rule [25]; this relates the sought-for cost to a known cost for that type of equipment and the ratio of their capacities raised to an exponent (0.6). However, Remer and Idrovo [30] warned against blind use of the exponent value 0.6 and presented exponential scaling factors for 58 different types and sizes of bioprocess equipment where the exponent value ranges from 0.36 for computer-controlled fermenters to 1.00 for ultrafiltration rigs.

3.2. Predicting COG

Manufacturing costs typically comprise direct production costs, such as raw materials and utilities, and indirect costs such as depreciation and insurance. The distinction between variable and fixed costs is not consistent in different sources; for example, labour can be considered to be either directly related to operating activities or to be a fixed annual charge. In addition, for cost analyses in the literature it is not always clear what is included under different cost categories.

Chemical and biochemical engineering textbooks (e.g. [25,31,32]) provide methods for estimating the cost categories that make up the manufacturing cost. The methods are based on calculating the direct operating costs from a process flowsheet. The remaining costs are calculated as percentages of the direct operating labour or fixed capital investment. These estimates are based on either chemical plants or traditional fermentation processes. They, therefore, provide useful concepts but do not account for the extra running costs in GMP facilities. Monitoring the direct utilities cost does not account for the ongoing utility charges for running the manufacturing facility. For example, HVAC systems are critical to controlling air particulate levels and air pressure differentials in different rooms so as to prevent contamination and securely contain micro-organisms used for production. It has been suggested that an extra cost category termed "general utilities" can be added to account for this [33], calculated as a function of the floor area. In addition to costs arising from the process flowsheet, it is also important to capture cost items incurred from ancillary activities, e.g. cleaning equipment and regulatory support activities such as documentation and quality control [33]. In traditional costing techniques, these costs may be hidden in overheads or unaccounted for.

Current economic evaluations may not account for hidden costs in biopharmaceutical manufacture. The time and cost issues of validation and regulatory issues are usually underesti-

mated. Another area of ongoing cost is environmental monitoring to maintain controlled environments. Pugh [34] highlighted further hidden costs including process development effort, batch failure, risk, change-over time, learning curve effects and plant utilisation versus product demand.

4. Software tools for process economics

Process economic models typically address strategic issues such as capital investment decisions, COG analysis, cash flow analysis, project management and risk assessment. Several approaches exist for performing economic evaluations of batch biotech processes. In order to determine the process COG, it is necessary to integrate process models with cost models [33]. Process modelling typically involves solving a combination of design equations and mass and energy balances so as to describe the technical performance of unit operations. Key factors that feed in from process models to COG models are the utilisation of key resources (e.g. materials, utilities, labour) and a measure of overall throughput or output (e.g. annual number of projects completed or annual kg output) [35–37]. Selecting a suitable software platform(s) for these combined process and cost models will depend on the requirements specification for the tool, the level of detail required and the desired outputs. Key choices include whether the model will be static or dynamic and deterministic or stochastic. These are discussed in more detail below.

4.1. Static versus dynamic models

Static models are often spreadsheet-based and are relatively simple and quick to build. Static process models can easily be linked to COG models to determine the cost breakdown and sensitivity of the COG to key process parameters. These can also be linked to cash flow models to determine the profitability of investment alternatives. Static models are particularly useful at early stages of a project where ballpark cost estimates are required. However, if a model has several interconnected worksheets it can be difficult to manage and update. Static models cannot account for situations where delays occur as a result of resource constraints; hence they are best suited for when such delays are not critical [38].

On the other hand, dynamic models relate to time-dependent operations and discrete-event simulation techniques, in particular, have gained popularity for modelling dynamic workflows and the logistics of operations. Discrete-event simulation models comprise activities that compete for resources; hence such models can capture parallel events and delays occurring during manufacture due to resource constraints (e.g. [38–40]). Discrete-event simulation models tend to be more complicated to build [38], but provide a more realistic production schedule [38–40]. Consequently, such models allow for more accurate estimates of throughput and hence cost. Puich and Paz [38] provide a useful summary table highlighting the key differences between static and dynamic modelling.

A further dimension that impacts on the type of model built is the level of detail required to determine the process outputs. For example, the annual kg output that feeds into the COG model can be determined using short-cut yield-based mass balance models or more rigorous models based on differential equations. The latter dynamic models, e.g. to model fermentation kinetics or chromatography adsoption kinetics, are usually set up in general purpose simulators such as MATLAB® (The MathWorks Inc., Natick, MA, USA) [41].

Process and cost models are not always implemented in the same software package. Dynamic process models to capture logistics and delays are often best suited to discrete-event software packages, whereas COG and cash flow tables are often best viewed in spreadsheet-based software (e.g. [40]). In addition discrete-event packages often facilitate linkage to spreadsheet packages to act as a graphical user interface for data entry and reporting [42]. Consequently, several examples in the literature can be found where discrete-event simulation models are linked to spreadsheet-based software (e.g. [37,43–46]).

4.2. Deterministic versus stochastic models

Traditional process modelling and investment analysis techniques assume all outputs occur with certainty and hence they are ‘no-risk’ performance measures. However, manufacturing decisions are often made in an uncertain environment characterised by technical and market-related risks. For example, common uncertainties in biopharmaceutical manufacturing systems include fermentation titres, purification yields, processing times, batch failures, and product demands [35,37,47].

Various approaches for identifying and measuring the uncertainty associated with a project appraisal have been advocated in the literature. The simplest method is to conduct a sensitivity analysis of each of the principal variables; the impact of $\pm x\%$ changes in each variable on the key output measures is observed. This provides a result for a given change, but it does not consider the likelihood of this change occurring [48].

More formal methods of incorporating risk require a subjective assessment of the probability distributions of all the key variables. Two of the methods for using this information are ‘risk adjustment’ and ‘Monte Carlo simulation’.

For risk adjustment, each input is weighted by the likelihood of occurrence. The key output measures are therefore risk-adjusted values that represent expected average values taking account of all possible outcomes. This method is recommended by Moilanen and Martin [48] for financial evaluation of environmental investments. Monte Carlo simulation techniques are used increasingly in various business situations [49]. This uses the input probability distributions to determine the resulting probability distributions of the outputs. These can then be used to identify the range of possible outcomes and the likelihood of exceeding a critical threshold value. The popularity of Monte Carlo simulation can be attributed to the increase in computing power. In addition commercial packages for Monte Carlo simulation have been introduced that are relatively easy-to-use and inexpensive; examples include the spreadsheet add-ons @RISK (Palisade Corporation, Newfield, NY, USA) and Crystal Ball (Decisioneering, London, England). Determining the probability distributions of the key uncertain inputs is usually based on historical data or subjective estimates from industrial experts. An

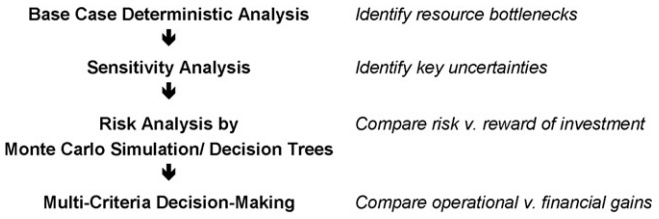

Fig. 1. A systematic framework for incorporating uncertainties in process economics studies.

increasing number of companies use subjective estimates where typically three levels for each variable are specified—minimum, most likely and maximum [49]. Booth [50] highlighted the importance of incorporating project-specific risks into investment appraisals that use discounted cash flow techniques. Cash flows are given appropriate probabilities and Monte Carlo simulation can be used to calculate the expected NPV. Booth [50] highlighted that project-specific risks should not be used to influence the discount rate. A systematic framework for incorporating uncertainties in process economics studies is summarised in Fig. 1 [35,47].

4.3. Commercial biotech process simulation packages

The commercial batch simulation packages reported to be used most widely for bioprocess modelling are SuperPro Designer (Intelligen Inc, Scotch Plains, NJ, USA) and Aspen Batch Plus (Aspen Technology Inc, Cambridge, MA, USA) [51]. A detailed evaluation of the suitability of the two packages for modelling a vaccine manufacturing process is provided by Shanklin et al. [52] Both packages handle material and energy balances, equipment sizing and costing, and economic evaluation. The fact that all these features are incorporated into one package, and hence can be investigated simultaneously, is a key advantage of these tools [52]. Further advantages include the graphical representation of flowsheets, default input values, the cost and profitability estimates and the ability to rapidly scale-up/down equipment sizes for different annual outputs [36]. Although both packages represent important tools for economic analysis, they have certain limitations [33,36,52,53]. Shanklin et al. [52] report that neither package accounts for constraints such as available labour or time limitations between cleaning and processing. These conclusions are echoed by Farid et al. [33] who reported that SuperPro Designer does not account for dynamic resource allocation or the impact of delays due to resource constraints and hence has limited logistical capabilities. Furthermore, these packages do not fully capture the resource utilisation associated with cleaning and sterilising operations [52]. Additional limitations are that they offer only pre-specified functionality with no option to create user-defined models [33,36,52] and do not allow the use of probability distributions to represent the uncertainty in parameter values [36].

5. Antibody process economics case studies

Having reviewed the methods for cost estimation and their relative uses and limitations, the following text provides an overview of the cases found in the literature that involve economic analyses of antibodies produced using different expression systems.

5.1. Mammalian cell culture

5.1.1. Process characterization studies

SuperPro Designer has been used to evaluate the economics of typical monoclonal antibody production processes based on mammalian cell culture [54–56]. Each provides details on the flowsheets and estimates of the capital investment, production cost and key profitability indicators. Harrison et al. [54] illustrate the sensitivity of the production cost to the annual production rate and demonstrates how the production cost drops, considerably, in what appears to be an exponential fashion, as the production rate increases 10-fold. The ratio of upstream to downstream costs is also shown to be sensitive to the annual production rate; for a production rate of 6.2 kg/year and a titre of 0.1 g/L the ratio is given as 46:54 and for a production rate of 100 kg/year and a titre of 0.5 g/L it is given as 20:80. However, it is not clear what categories are included in these costs. Harrison et al. [54] also highlight that for large outputs of 100 kg/year, switching to alternative expression systems, such as transgenics, will therefore only impact 20% of the production cost, unless less expensive purification technologies are also developed. Petrides and Siletti [55] comment that increasing productivity by adding an extra fermenter can cause a slight increase in the unit production cost if the added capacity is just used to make the same number of batches in a shorter time; but if the increased capacity is used to make extra product, the production cost drops in the example provided. Oh et al. [56] also used SuperPro Designer to evaluate the impact of adding fermenters operated in staggered mode to double the annual production rate. They provide a detailed breakdown on the investment costs, production costs and the profitability analysis for the base case and the optimised case with increased capacity. The actual costs derived for the capital investment and unit production costs appear to be much higher than the benchmark data collected in Section 2. Their results illustrate that although the increased capacity caused a 12% increase in the total capital investment and an 88% increase in the annual production cost, the unit production cost drops by 7% and the ROI increases by 78%. Of course the impact of adding extra capacity will depend on the scale, how many fermenters are added and how much additional product is made as a result.

Considering the distribution of costs across each activity can provide further insight on where to focus process development efforts. For a conventional 200 L antibody process, a breakdown of the direct COG/g on an activity basis suggests that the Protein A affinity chromatography step is the most expensive step owing to the high cost of the matrix at $7500/L [35]. Hence, it has often been quoted that larger bioreactor scales of, say, 10,000 L operating with a titre of 1 g/L, may result in Protein A matrix costs of $4–5 M [40]. These high material costs have led to suggestions that Protein A matrices be used in smaller quantities with multiple cycles and be reused despite the complications of reuse validation [40].

Sommerfeld and Strube [5] explore the potential to optimise monoclonal antibody processes. No details on the models or software used are given. However, the authors provide useful insights on how different parameters affect the cost of goods. They calculate that increasing the fermentation titre 10-fold from 0.1 to 1 g/L causes the ratio of upstream to downstream costs to drop from 55:45 to 30:70. Consequently they suggest that downstream processing costs are a major cost factor at 1 g/L titres and therefore offer the largest optimisation potential. They show that there is potential to optimise expensive affinity separation steps if the binding capacity of the resins can be increased. Such a change obviously results in less resin being needed which lowers the consumables costs which are quoted to represent up to 49% of the downstream production cost. However, they found that increasing the binding capacity of ion exchange resins has less impact on the operating costs as the resins are much cheaper; a trade-off exists between the drop in consumables cost and the increase in labour costs which becomes more dominant at higher binding capacities due to the longer processing times. They also comment that they were surprised at the high cleaning costs for the membrane separation steps and indicated that this would be another worthwhile area for improvement. More details on the assumptions made, such as the scale of the process or the production rate (kg/year), would make it easier to interpret some of the results and compare with other studies.

5.1.2. Facility decisions

Further publications compare alternative ways to manufacture monoclonal antibodies using mammalian cell culture. Facility decisions such as exploring the use of disposable components are addressed [4,35,44,57,58]. Sinclair and Monge [44] provide a detailed study of the impact of disposable bag technology for media and buffer preparation, as well as for media, buffer, and product hold in a 2000 L process. The impact on utility systems and CIP systems are analyzed using discrete-event models and the subsequent effect on the cost of goods computed using spreadsheet models. They found a 21% decrease in capital investment costs and a 9% decrease in cost of goods compared to using stainless steel vessels. This study was extended to look further at the impact of the use of single-use bags and coupling connection technologies on the facility design and footprint. This was applied to a 1000 L perfusion-based process and resulted in a 41% reduction in the capital costs and a 17% reduction in the COG/g.

A further study [4,35] presents a hypothetical case evaluating the use of a fully disposable plant for preparation of antibody candidates for early-phase clinical trials. Although the main purpose of the case study was to demonstrate the application of a framework for evaluating the cost and riskiness of different manufacturing strategies, it provides an indication of the possible consequences of adopting disposables for upstream and downstream operations. Here stainless steel fermenters (up to 200 L) were replaced with disposable versions and downstream consumables such as matrices and membranes were used in a disposable fashion. The study assumed media and buffers arrive pre-made and pre-sterilized and hence no media/buffer preparation steps were modelled. However, the more decisive equipment preparation steps such as cleaning and sterilization were modelled explicitly. The activities, resources, process streams, costs and uncertainties were captured in a discrete-event model set up in the object-oriented platform ReThink v3.1 (Gensym Corporation, Cambridge, MA, USA). A sensitivity analysis indicated that the critical driver affecting the annual COG/g was the fermentation titre. This is to be expected since the titre significantly affects the production rate and hence productivity. The authors found that the degree of uncertainty in the resource costs has relatively minor impact on the outputs. The effects of fluctuating product demands and titres were analyzed using Monte Carlo simulations. This added an extra dimension to the project appraisal as investments could be assessed based on both the expected outputs and their associated risks. In this particular hypothetical case, the use of disposables for Phase I material production at the 200 L scale was shown to be favorable under certain scenarios that assumed similar titre and yields as conventional processes. Critical drops in these drivers that affect the decision were also identified.

The analysis was also taken a step further to account for both quantitative and non-quantitative parameters that ultimately need to be considered such as the construction time, the flexibility to modify process configurations, reliance on suppliers and the trade-off between the validation effort for cleaning in a stainless steel plant and for extractables leached from plastics in a disposable plant [4,57]. A stochastic additive weighting technique was used to capture and aggregate the financial and operational attributes under uncertainty. The model was set up in Microsoft Excel using the Excel add-on @RISK (Palisade Corporation, Newfield, NY, USA) to perform the Monte Carlo simulation technique. This study predicted that a conceptual 200 L disposables plant could potentially provide financial and operational savings for supplying early phase material. Some of the drawbacks of disposables such as the increased reliance on suppliers and scale-up complications become of prime importance during Phase III clinical trials and commercial manufacture and hence their weightings were adjusted to reflect this. The analysis indicated that if financial savings are considered a more significant driver, the disposable option still outperforms the more expensive stainless steel option. On the other hand, if the operational benefits are considered more significant than financial savings, these drawbacks result in the stainless steel option competing with the disposable option for supplying late-stage clinical material. However, the authors highlight that it is common for start-ups to be bought out by larger pharmaceutical companies who possess their own technology and so these drawbacks may not be crucial to a start-up's success. Although disposables appear cost-effective at the 200 L scale, it would be interesting to explore whether disposables still prove economic as scales increase beyond this level. It has been reported that downstream processing using disposables becomes a major disadvantage at the 10,000 L scale [12].

5.1.3. Upstream process decisions

Upstream process decisions explored in the literature focus on issues related to fed-batch or perfusion culture. Lim et al. [46]

assessed pooling strategies with perfusion culture processes. The analysis addresses the trade-offs between investing in a plant with a smaller downstream processing (DSP) capacity and employing more frequent pooling of the broth for purification or opting for a plant with a larger DSP capacity and less frequent pooling of broth. In this paper, a risk-based discrete-event prototype tool built in Extend Industry Suite v5 (Imagine That Inc., San Jose, CA, USA) was used to incorporate the upstream and downstream operations as well as the ancillary activities related to equipment preparation and regulatory compliance activities, such as QCQA and batch documentation activities. The key performance metrics were the expected annual output, the probability of failing to achieve a certain output, the expected COG/g and the associated risk. In this particular case study, shorter pooling intervals were shown to be more favourable when the perfusion process was subject to small variations in titre and yield. The impact of the stability of product and downstream equipment scale were also highlighted in further scenario analyses.

An extension to the above-mentioned analysis used the same tool to evaluate the process economics of fed-batch and perfusion culture for the commercial production of antibodies [37]. The trade-offs between the lower productivities and higher upfront investments of fed-batch processes versus the greater operational risks with perfusion processes are analysed. Interestingly, the deterministic simulation results illustrated that both the fed-batch and perfusion processes have similar cost of goods per gram, under the assumptions made in this particular case study. However, since the perfusion option offers lower initial investment costs and hence a higher projected NPV, the deterministic analysis predicts that this mode of operation is more economically feasible than the fed-batch option. However, when accounting for fluctuations in titre, DSP yield and the possibilities of contamination and equipment failure, the Monte Carlo simulation results demonstrated that the perfusion option has a lower reward/risk ratio and fails to meet the desired output criterion. The analysis identifies how low the probabilities of contamination and bioreactor failure due to filter fouling must drop for the perfusion option to be more favourable than the fed-batch option. Of course these results are specific to the assumptions made in the case study which initially assumed the chances of bioreactor failure were 10%. New designs of perfusion reactors mean that the chances of filter fouling can be much lower making perfusion culture a cost-effective option with its lower upfront investment. However, it is recognized that the choice between fed-batch and perfusion processes often depends on the experience within the company [59].

5.1.4. Downstream process decisions

The remaining contributions in the literature tend to focus primarily on the cost of chromatographic separations rather than whole processes. A useful analysis was carried out by Fulton et al. [60] that compared the use of conventional chromatography with perfusion chromatography operated in two cycling modes for the separation of monoclonal antibodies, tPA and animal growth hormones. The authors indicated that capital and operating costs were projected using conventional estimation methods, giving an accuracy of ±30%. The cost savings, operational benefits from cycling with perfusive columns and the potential disadvantages of such an approach are highlighted.

The cost-effectiveness of switching from conventional product recovery to expanded-bed chromatography (EBA) in antibody processes has been explored [61]. The case investigated the adoption of EBA, in combination with a microfiltration step, to replace four unit operations: two microfiltration steps, an ultra-/dia-filtration step, and a packed bed chromatography step. The results indicate that for this particular scenario, downstream operational costs are reduced by 65–70% despite an increase in matrix cost of 30–60%. However, the analysis did not assess the full impact on the whole process cost.

Dowd and van Reis [62] present insights from studies to reduce recovery and purification costs using models built in Excel. Interestingly they report that the labour costs for buffer preparation operations are almost equal to those for the downstream unit operations. They comment that pooling intermediate batches can produce significant labour and quality savings.

5.2. E. coli

Although monoclonal antibodies and antibody fragments can be considered as different products it is still useful to consider the possible costs implications of alternative expression systems such as *E. coli*. Novais et al. [11] provide a detailed economic analysis of the use of disposables-based processes for the commercial production of antibody fragments produced in *E. coli*. A 300 L bioreactor capacity was assumed with the flowsheet based on the assumption that the fragment is expressed in the perisplasmic space, thus requiring lysis prior to the purification by chromatography and filtration. Costing models were developed which permit the investment cost of disposables-based facilities to be approximated based on the equipment cost in a conventional facility; Lang factors of 8.1 and 4.7 were derived for the conventional and disposables-based plants, respectively. NPV analysis was used to assess the viability of disposables under different scenarios with different times-to-market, yields and costs. Since the running costs were derived based on percentage breakdowns provided by Datar et al. [63] for bacterial processes involving inclusion bodies, they cannot be compared directly with those reported for mammalian cell cultures. Datar et al. [63] compared the costs of producing the recombinant protein, rtPA, in *E. coli* and CHO cells, and illustrated how the expression system could have a major impact on the total number of required processing steps and hence the economic viability of a product. In their case, the extensive downstream processing required to refold the inclusion bodies meant that the CHO cell process was more economically feasible. However, more studies are needed to see whether *E. coli* production costs for antibody fragments directed to the periplasm are lower than mammalian costs owing to the simpler fermentation media used and shorter fermentation times while retaining similar downstream processing steps.

5.3. Transgenic organisms

Detailed attempts at deriving costs for transgenic expression technologies have also appeared in the literature, anticipating ton-scale production of antibodies. When comparing transgenic systems with cell-culture-based processes, any reported savings are realised during the upstream processing; the costs during downstream processing are similar since the same purification methods are used. Young et al. [64], Fulton [65] and Onigman [13] present a promising outlook for the economic potential of transgenic goats based on experience at Genzyme Transgenics (Framingham, MA, USA). Young et al. [64] illustrate that for an annual demand of 100 kg of antibodies, 35 goats with expression levels of 8 g/L/day are equivalent to an 8500 L cell culture reactor with a 1 g/L titre after a 10-day cycle. This results in almost an order-of-magnitude reduction in the litres that need to be purified. The authors suggest that transgenic dairy animals require lower upstream capital and running costs than cell culture facilities since the husbandry and dairying costs, similar to standard agricultural costs, are lower than the costs associated with GMP cell culture facilities; they conclude that antibody production would cost approximately $100/g compared with $300–$3000/g with mammalian cell culture. The influence of annual production output on the cost of goods and capital investment are highlighted for different expression levels and purification yields. For example, they illustrate how the transgenic cost of goods drops from $100's/g to $10's/g as the annual production capacity increases from 10 to 1000 kg. Fulton [65] provides a cost breakdown of the total material, labour, overhead and depreciation costs for the production of monoclonal antibodies in transgenic goat's milk at a production output of 1000 kg/year. For all these analyses, more detailed information on assumptions made would be helpful in understanding how the capital and running costs were deduced—e.g. the pooling strategies adopted to cope with the daily milk production, the impact on the size of the purification suites and the purification costs.

Mison and Curling [66] assessed the costs of producing a recombinant protein in transgenic corn based on pilot scale experience at Meristem Therapeutics (Clermont-Ferrand, France). A clear account is provided on how the costs were derived at different expression levels, purification yields and plant capacities using Microsoft Excel spreadsheets. The impact of increasing annual outputs from 0.1–100 ton on the COG/g and the cost components is highlighted. They found that the capital-related and labour costs dominate at low outputs but become less significant as the annual output increases to 100 ton/year; in contrast the raw material costs rise considerably from 14 to 67% of the COG/g. Thus, economies of scale result in a disproportionate effect on raw materials. Similar trends have been reported for cell culture processes as output increases [67]. Consequently raw materials savings become more important for any process as scale increases. The authors also illustrated how the results of sensitivity analysis to individual factors could be combined to determine the overall adjustment factor to apply to the production cost as a result of multiple factors changing values. Finally the authors compare the transgenic plant production costs with other expression systems and conclude that transgenic plants offer the cheapest route. Similar conclusions of order-of magnitude reductions in COG/g have been reached by other authors [68]. Others have predicted less dramatic savings with transgenic plants of 20–40% [69]; these differences may be attributed to different assumptions of the ratio of upstream to downstream to quality costs.

Sinclair et al. [70] argue that transgenic chicken systems offer more cost-effective production of monoclonal antibodies than transgenic mammals or plants. They provide an economic comparison of the cost for goods and NPV for producing 100 kg/year of an antibody using transgenic chickens and mammalian cell culture. At this scale they found a 55% reduction in both the cost of goods and NPV with transgenic chicken. Through NPV analysis they were able to also take into account the costs of development in each case and the times required to establish a flock versus the time required to construct a fermentation plant. Based on the assumptions that these costs and times are halved in the transgenic chickens case, the transgenic chicken option allowed for later investment when product risk is reduced; consequently the authors highlight that this will minimise losses in the event of product failure.

As mentioned earlier, given the recent increase in antibody titres possible in mammalian cell culture, with values of 5 g/L reported [2,6,14], it would be interesting to see the impact on the comparisons between conventional cell culture and transgenic options.

5.4. Antibody portfolio analysis

The influence of antibody manufacturing activities on the management of R&D portfolios has been assessed using NPV analysis [43,71,72]. Such analyses measure manufacturing using measures such as the overall productivity, COG/g or COG/batch and include other parameters such as the dosage, probability of clinical success, time spent in development, contracting out manufacture, time for building facilities and learning curve effects.

6. Future outlook

All marketed antibodies are currently made by mammalian cell cultures [3]. Given the high doses and the increasing market potential of therapeutic antibodies, new approaches may be needed to be capable of producing the 10–100 s kg/year of antibodies at lower costs. Significant challenges exist with all the currently available options. Increasing use of disposables not just for media and buffer preparation but also for bioreactors and downstream processing is becoming a reality. For inoculum preparation and clinical trial material preparation, it is becoming more common to see stirred tank bioreactors replaced by disposable bioreactors such as the Wave bioreactor (Wave Biotech, NJ, USA) (up to 500 L) or reactors with plastic bag liners [6,73,74]. However, the scale limitations of disposable bioreactors that currently reach 1000 L (e.g. the XDR disposable bioreactor (Xcellerex, Marlborough, MA, USA)) may limit their economic potential for commercial manufacture; at present it is not clear how disposables fare for drugs targeting large markets

and with high doses. Applying modular construction can also potentially save costs by simplifying construction and reducing the time to build [75].

The use of transgenic mammals, chicken and plants as culture systems is actively being pursued for antibodies with projected annual marketed demands of several hundreds of kilograms, if not tons. Despite higher productivities, competitive timelines and lower costs, significant regulatory and safety hurdles still need to be resolved. Antibody fragments are being engineered to enhance in vivo half-lives and have been produced successfully in *E. coli* and *Pichia pastoris* for candidates in preclinical and clinical trials. The processes used are similar to those used for mammalian cell culture derived products [3]. In *E. coli* additional steps are required for periplasmic extraction or cell lysis which can involve heat treatment [76]. With potential titres of up to 3–15 g/L [14] and shorter fermentation cycles, such systems are expected to drive down costs.

The case studies in the literature, as well as historical experience within companies, provide an indication of what drives the economics of cell culture processes. The overriding cost driver in producing monoclonal antibodies has been shown to be the bioreactor titre. Increasing cell culture titres of 3–5 g/L, which are expected to improve to 10–15 g/L in the next decade [14], are driving the search for novel approaches in downstream processing to ensure the purification costs do not negate the cell culture gains. Strategies include the use of expanded bed chromatography, synthetic affinity ligands, rigid chromatography matrices that allow for higher operational flowrates, membrane chromatography and crystallisation. However, with each of these there are trade-offs that need to be evaluated to assess the impact on process economics. Further potential to improve the process economics include reducing the number of downstream steps; e.g. it may be possible to avoid buffer exchange steps by designing each chromatography step so that it can take the material eluted from the previous step [22,74].

These issues are of immediate concern to an industry facing a fast rate of developments upstream and will need to be reflected in equally radical downstream processing solutions. The capacity to cost such alternatives does provide a common basis for such decision-making and as such will prove a vital tool for bioprocess designs in the future.

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