Process economics evaluation of cell-free synthesis for the commercial manufacture of antibody drug conjugates

Christos Stamatis[a] and Suzanne S. Farid[a,*]

[a]The Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, University College London, Gower Street, London WC1E 6BT, UK

[*]Corresponding author:

Prof. Suzanne S. Farid,

Department of Biochemical Engineering, University College London, Gower Street, London WC1E 6BT, UK

s.farid@ucl.ac.uk. Tel: +44 20 7679 4415

Abstract

Continuous improvements of cell-free synthesis (CFS) systems have generated interest in adopting the technology for the manufacture of biologics. This paper provides an evaluation of the manufacturing cost-effectiveness of CFS for the commercial production of antibody-drug conjugates (ADCs). The evaluation was performed using an advanced techno-economic engine (TEE) built in Python. The TEE is programmed in an object-oriented environment capable of simulating a plethora of process flowsheets and predicting size and cost metrics for the process and the facility. A case study was formulated to compare the economics of whole bioprocesses based on either a CFS system or a mammalian cell system (CHO) for the manufacture of an ADC at a range of product demands. The analysis demonstrated the potential of CFS for the commercial manufacture of biologics and identified key cost drivers related to the system. The CFS system showed an approximately 80% increase in the cost of goods compared to CHO with a significant cost attributed to the in-house manufacture of the bacterial cell extract, necessary for the CFS reaction step in the process. A sensitivity and target analysis highlighted the need for further process improvements

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especially in the titre for the CFS process to become more competitive against well-established systems.

Keywords
Antibody-drug conjugates, cell-free synthesis, manufacture, process economics, techno-economic analysis

Abbreviations: CFS, cell-free synthesis; TEE, techno-economic engine; CHO. Chinese hamster ovary; ADC, antibody-drug conjugate; COG, cost of goods; COG/g, COG per gram of product;

Introduction

Successful commercialisation of new biologics will require disruptive and integrated manufacturing technologies. Currently, commercial protein-based biologics are manufactured predominantly using cell culture processes with extended production cycles that typically span 2 – 3 weeks [1-2]. Cell-free synthesis (CFS) has emerged as an alternative to cell-based systems for the commercial production of biologics within hours instead of weeks [3]. Therefore, systematic tools are required to assess the feasibility of such new technologies, identify process bottlenecks and key cost drivers, and determine the necessary process improvements to become cost-competitive. This research focuses on creating novel decision-support tools incorporating CFS as an alternative system for the manufacture of antibody drug conjugates (ADCs) to gain an understanding of the process economics of the system compared to a cell-based platform.

Typically, the commercial manufacture of therapeutic proteins is achieved using living cells taking advantage of cell proliferation and the intracellular machinery for the expression of the desired product. Using a living cell system usually requires extensive cell engineering and development effort to program the cells in delivering the protein of interest [1]. Therefore, cell-based methods are commonly associated with lengthy development and manufacturing timeframes [2].

CFS is an acellular system that combines several chemical and biological reagents to mimic *in vitro* specific biological functions [4-7]. Thus, due to the absence of a cell wall, monitoring and control of the process can be significantly improved [8]. Published work has evaluated the CFS system for the production of monoclonal antibodies (mAbs) [9], bispecific antibodies [10], ADCs [11-12], fusion proteins [13], peptides [14] and vaccines [13]. A recent review discusses the potential of CFS for different applications [15]. CFS requires certain reaction components: a cell extract, the DNA template with the genes of the protein of interest, and a mixture of building blocks and energy sources. Published work has demonstrated the use of microbial cell extracts (*Escherichia coli*) for the manufacture of non-glycosylated mAbs [9]. Moreover, recent advancements such as a simpler reaction mixture [9], reproducible protocols for the production of bacterial [16-17] and mammalian cell extracts [18-19] and demonstration of scalability [3, 20], combined with the potential to accelerate drug development and manufacture timelines [21-22], have triggered interest in the potential of CFS for the commercial manufacture of biologics.

Furthermore, CFS processes have been reported to confer quality advantages for certain molecules, such as ADCs for oncology indications. ADCs are targeted therapies that combine the targeting precision of a mAb with the potency of a cytotoxic drug (payload). To date, there are nine marketed ADCs: Adcetris® (Seattle Genetics), Kadcyla® (Genentech/Roche), Mylotarg® (Pfizer), Besponsa® (Pfizer), Polivy® (Genentech/Roche), Padcev® (Astellas/Seattle Genetics), Enhertu® (Daiichi Sankyo/AstraZeneca), Trodelvy® (Immunomedics), Blenrep® (GSK). The manufacturing processes for the first generation marketed ADCs involve non-selective conjugation of mAbs, derived from CHO processes, to the cytotoxic-linker payload. This often leads to heterogeneity in the ADC population in terms of conjugation sites and drug-to-antibody ratios (DARs), which could potentially impact the critical quality attributes of the final product [11]. The incorporation of non-natural amino acids through a CFS system has demonstrated a significant increase in ADC homogeneity through site-specific conjugation and a consistent DAR; this can translate into an improved product quality [11]. ADCs generated using CFS systems based on *E. coli* extracts are in clinical trials for oncology

indications, currently driven by Sutro Biopharma and its collaborators (e.g. Bristol-Myers Squibb). Hence this paper focuses on determining whether cost benefits can be added to the other sources of competitive advantages known to CFS, namely, speed to market, quality, and flexibility.

Process economics studies have been applied in the mAb sector to aid with the evaluation of alternative technologies such as continuous operations [23-26]. A recent review summarised a representative number of process economics studies [27]. However, there is limited published work on the economic evaluation of CFS. Recently, a techno-economic analysis evaluated mAb production with a CFS process using a CHO cell extract and concluded that CFS would need significant improvements in titre to reduce the cost of manufacture [22].

This paper focuses on the evaluation of the cost-effectiveness of a CFS system against a well-established cell-based system for the production of ADCs. The Department of Biochemical Engineering at UCL has a history of developing decision-support tools to address challenges in bioprocessing from the process (e.g. [28]) and the facility (e.g. [29-30]) to the portfolio level (e.g. [31]). The tool developed here builds on previous work at UCL at the process economics level with additional capabilities in simulating new technologies (i.e. CFS) and manufacturing strategies (e.g. parallel process flowsheets and in-house versus outsourcing manufacturing options). The following sections of this paper provide a high-level description of the structure of the techno-economic engine and describe the formulation of a case study to demonstrate its functionality by evaluating the potential of CFS. A techno-economic analysis is performed to compare the cost of goods per gram of product (COG/g) for the whole bioprocesses based on CFS versus a mammalian cell-based system (CHO) for the commercial manufacture of an ADC. In-depth analysis of the cost of goods (COG) breakdown identified key cost drivers to consider and evaluate in a sensitivity analysis. Finally, a target analysis is performed to determine the necessary process improvements for the CFS to become more commercially feasible and competitive against the conventional approach of using living cells for the production of biologics.

Materials and Methods

1.1. Model structure

An object-oriented simulation engine was developed in Python™ (v3.6), edited in Spyder (v3.2.6), and operated through Jupyter Notebook (v5.2.2). Figure 1A illustrates a schematic of the structure of the techno-economic engine and its main components. The simulation engine was linked to a database of default assumptions and unit costs as well as a user interface with the key inputs and interactive outputs that could be exported as reports in Microsoft Excel. The simulation engine guides the user inputs through a hierarchical structure from the definition of a therapeutic protein to the design of the required process flowsheets to the bio-manufacturing facility parameters. Key user inputs to the model include product parameters such as the annual product demand and target product profile in terms of impurity specifications to be met; flowsheet parameters such as the operating conditions, resource requirements and performance (e.g. titre), a ratio of upstream (USP) to downstream processing (DSP) trains; and facility parameters such as the operational time and shift pattern. The analysis starts with the mass balance calculations and the sizing of core process equipment (e.g. chromatography columns and skids) along with their ancillary equipment (e.g. buffer, media, and product hold tanks).

The design calculations for different unit operations were based upon previous work conducted at UCL Biochemical Engineering [32]. The economic metrics determined by the tool were the fixed capital investment to build the facility and the cost of goods per gram (COG/g). The fixed capital investment was estimated using the Lang factor method [33]. The COG/g captured both the direct (e.g. materials) and indirect (e.g. maintenance) costs as outlined in [32]. The cost of materials accounts for reagents (e.g. media, buffers, cleaning agents), consumables (e.g. filters, resins), and miscellaneous materials. Labour costs include the cost of operators, supervisors, management, and quality control and assurance personnel; the labour costs were calculated based on the number of operators per shift per USP and DSP train and their annual salary. The indirect costs considered

facility-related overheads such as depreciation, maintenance, insurance, and local taxes of the facility along with general utilities (e.g. HVAC).

The required working volume for the CHO cell culture, the CFS reaction, and the *E. coli* fermentation is calculated by the techno-economic engine as follows:

$$V_{working} = M \Big/ \left(B * T * \prod_{DSP} Y \right) \text{ (Equation 1)}$$

where $V_{working}$ is the required bioreactor working volume (L), M is the annual product demand (g), B is the number of batches, T is the titre (g/L) and Y is the yield of each step in downstream processing (DSP). New design calculations were introduced to simulate the CFS reaction step. Given the required working volume for the production bioreactor, the required volume of each main components in the CFS reaction was estimated as follows:

$$V_{Extract} = V_{working} * \nu_{Extract} \text{ (Equation 2)}$$

$$V_{T7RNAP} = SA_{T7RNAP} * C_{T7RNAP} * V_{working} / AA_{T7RNAP} \text{ (Equation 3)}$$

$$V_{DNA} = C_{DNA} * V_{working} / SC_{DNA} \text{ (Equation 4)}$$

$$V_{Master\ mix} = V_{working} - V_{Extract} - V_{T7RNAP} - V_{DNA} \text{ (Equation 5)}$$

where V is the volume (L), v is the volumetric concentration (v/v), SA is the specific activity (U/mg), C is the required concentration (g/L), AA is the available enzyme activity (U/mL) and SC is the stock concentration (g/L).

The simulation engine was linked with a Microsoft Excel 2016 spreadsheet containing values for assumptions for each unit operation and costs for different process equipment and materials. Table 1 summarises the key assumptions used by the techno-economic engine for the case study. Additional assumptions are summarised in the supplementary tables (Table S1 – 3). Furthermore, the engine was imported and operated in Jupyter Notebook through the development of a user-

interface to manipulate and control different segments of the engine. The outcome of a simulation was exported and stored into a different MS Excel spreadsheet for further analysis and visualisation.

1.2. Scenario analysis formulation and key assumptions

To evaluate the cost-effectiveness of a CFS system relative to a CHO system, a case study was formulated assuming a single-product facility for the commercial manufacture of an ADC. The case study explored the trade-offs between the faster generation of products with CFS systems and the higher productivities versus the additional costs to produce the cell extract for the CFS reaction relative to the CHO system. More specifically, the bioreactor for the CFS batch reaction ran for 14 hours with a 1g/L titre in contrast to the CHO fed-batch cell culture that ran for the typical 14 days with a fairly conservative 3g/L titre. This translated into an 8.5-fold higher volumetric productivity for CFS of 1.7g/L/day compared to 0.2g/L/day for the CHO system.

Estimated demands for current ADCs based on sales reported suggest current annual demands in the order of tens of kilograms, with some future projections suggesting demands may reach the low hundreds. Hence, a range of annual product demands (25, 100, and 175kg/year) was investigated to determine how a CFS system compares with a CHO system. Regardless of the scale of manufacture, it was assumed that reusable process equipment was used throughout the process (e.g. bioreactors, filter housing units, tangential flow filtration skids, chromatography columns, etc.). On the other hand, depth filters and virus removal nanofilters were considered single-use and discarded after every batch. Media and buffers were assumed to be purchased pre-made from a third party vendor. Finally, the manufacturing costs were estimated for the drug substance and not for the drug product thus excluding the final fill-finish step.

The process steps and the flowsheets for the manufacture of an ADC using the CHO and the CFS systems along with the process steps for the in-house supply of the *E. coli* cell extract to the CFS process are shown in Figure 1B. For this case study, the performance of the individual DSP steps was assumed to be equivalent between the CHO and the CFS process (Figure 1B) due to the lack of

published data that could support the opposite. The exception is the conjugation step yield (72%) for the CFS process which was assumed to be equal to the overall yield of the modification (90%) and conjugation (80%) steps of the CHO process (Figure 1B). Using the CHO system, the conjugation reaction was modelled as a two-step process starting with the addition of the linker to the mAb followed by the addition of the cytotoxic drug. However, using the CFS system, it has been reported that the incorporation of non-natural amino acids could potentially remove a process step by using an integrated linker-cytotoxic molecule [11]. The small difference in the overall DSP yield between the CHO and the CFS processes (42% and 45%, respectively) is due to the absence of the virus inactivation and the virus filtration steps from the CFS process since there is not a mammalian cell line involved with the CFS process.

For the CHO and the *E. coli* processes the flowsheets start with a series of shake flasks followed by the seed bioreactor train to prepare the inoculum for the production bioreactor. In the context of this study, shake flasks and seed bioreactors trains are described as the inoculum grow-up and seed train step. The *E. coli* process flowsheet for the extract was simulated in parallel with the CFS process flowsheet and it was assumed that a single *E. coli* batch could be allocated to multiple CFS batches. In this case study, it was assumed that a single *E. coli* cell extract batch could supply two CFS batches to minimise the hold and storage time for the extract and balance the difference in the batch time between the CFS and the *E. coli* processes. The bioreactor for the CFS reaction was assumed to be operated in batch mode while the CHO cell culture and *E. coli* fermentation to generate the cell extract were simulated in fed-batch mode with a volumetric ratio of base to feed media of 3:1.

For the economic evaluation of the CFS system through the analysis, a set of assumptions were considered using already published work for the operation of a CFS reaction step. To determine the impact of each assumption on the cost-effectiveness of the manufacturing process a one-way sensitivity analysis was performed (Python™ and Jupyter Notebook) using the worst and best case values for each assumption in Table 1. The main objective of the sensitivity analysis was to rank

process parameters related to CFS based on their impact on the COG/g and to identify the parameters with the greatest potential to lower the COG/g. Additionally, the parameters with the greatest influence on COG/g as identified through the one-way sensitivity analysis were challenged simultaneously to their best-assumed values to determine the combined COG/g reduction that can be achieved. Finally, the sensitivity analyses were extended to a set of 2-way sensitivity analyses shown as contour plots across a matrix of different starting assumptions; the windows of operation that enabled the CFS process to reach similar COG/g values with the CHO process were identified.

Results and Discussion

This section presents insights from the cost of goods analysis comparing CFS with CHO for the commercial manufacture of an ADC. A sensitivity analysis is then used to identify critical model parameters that impact the cost of goods for the CFS process. Furthermore, a target analysis is presented to determine what process improvements are required for the CFS process to become cost-competitive with CHO.

1.3. Manufacturing scale

CFS has demonstrated significant improvements over the past five decades with the biopharmaceutical industry evaluating and developing the technology for the commercial manufacture of biologics. To determine the cost-effectiveness of a CFS system and compare it with a CHO system, the metric COG/g was used as a comparator.

A summary of the key sizing and operational results is provided in Table 1. Using the CFS system a relatively large number of batches can be achieved with a single USP train due to the significantly faster upstream processing time compared to a CHO system. With the CHO process, a maximum of 20 batches can be performed in 330 days. The CFS process can fit 66 batches within the same facility operational time.

The speed of a CFS process allows for a greater number of batches that would typically translate into a smaller bioreactor size with the condition of titres being equivalent. However, the lower titre of the CFS reaction compared to the CHO cell culture limits any significant benefits in process equipment sizing. For instance, to achieve a target demand of 100kg/y, a bioreactor working volume of around 4,000L and 3,400L is required for the CHO and the CFS process, respectively. Additionally, 33 *E. coli* batches were performed to supply the required cell extract using a bioreactor of 3.800L.

1.4. Cost of goods breakdown

A breakdown of the COG/g for each system is presented in Figure 2A. The breakdown in Figure 2A categorises costs like labour, materials, and facility-related. The costs related to the CFS system consider the process for the manufacture of an ADC and the *E. coli* process for the manufacture of the cell extract.

The COG/g values for a CFS system are approximately 65 – 85% higher compared to a CHO system for the manufacture of an ADC across different product demands (Figure 2A). Hence, although CFS offers higher productivity, this did not translate into a cost reduction under the current scenario configuration. Others [22] have reported higher cost differentials between CFS and CHO processes when using a CHO cell extract.

The significant cost increase associated with CFS can be mainly attributed to the in-house manufacture of the *E. coli* cell extract. The value of COG/g of ADC attributed to the *E. coli* process is 675$/g to 195$/g at 25kg/y to 175kg/y scales, which represents approximately 30% of the COG/g. Additionally, the unit cost of the in-house manufacture of the *E. coli* cell extract is 50$/g to 15$/g of extract as the demand for ADC increases from 25kg/y to 175kg/y. Although the unit cost for the manufacture of the *E. coli* extract is relatively low, the large volumes needed lead to a significant increase in the COG/g for the manufacture of an ADC using the CFS process. Therefore, additional improvements to reduce the extract requirements are necessary to bring down the COG/g. The

impact of process parameters related to the *E. coli* cell extract on the COG/g for the manufacture of an ADC is further explored in the following sections to determine the key improvements needed.

1.5. Key cost drivers

The key cost driver across different product demands is the cost of materials for both the CHO and the CFS system (Figure 2A). Thus the next stage was to determine which process stages and unit operations were contributing the most to the cost of materials. Focusing on the cost of materials, Figure 2B presents a breakdown among the main stages of the process at 100kg/y demonstrating the portion of the cost of materials due to the in-house manufacture of the *E. coli* extract. Almost a quarter (27%) of the cost of materials for the CFS system is due to the *E. coli* extract process. Furthermore, chemical and biological reagents dominate the cost of materials throughout. Additionally, Figure 2C shows the distribution of the cost of materials among the unit operations for the CHO and the CFS processes including the extract process flowsheet at 100kg/y. The percentage cost of materials for the production bioreactor (cell-free protein synthesis step) increases from around 20% for the CHO process to 60% for the CFS + Extract process. A breakdown of the materials cost of the production bioreactor for the CFS process shows that materials involved in the extract manufacture account for 47%. The other CFS reaction components account for 44% (master mix 22%, T7 RNA polymerase 15%, and DNA template 7%) with the remaining cost attributed to cleaning reagents (9%). Finally, the pie chart in Figure 2C shows a breakdown of the cost of materials per process step for the manufacture of the extract with the sum of fermentation and homogenisation materials accounting for ~65% of the cost of the *E. coli* process materials.

The cost breakdowns in Figure 2C demonstrate the significant contribution of the *E. coli* cell extract process to the overall COG/g for the ADC using the CFS process. Hence, it is important to identify the key parameters related to the CFS reaction step that could have a significant impact on the resource consumption and equipment sizing to determine the focus of further process development and reduce the COG/g. This is explored in the next sections.

1.6. Sensitivity analysis

The deterministic analysis of a CFS system was based upon certain assumptions regarding its operation and associated costs (Table 1). To identify the impact of several key assumptions related to the CFS reaction step on the total COG/g, their base values were challenged to worst and best cases (Table 1). Figure 3A illustrates a tornado graph visualising the impact of key CFS assumptions on the COG/g. The significant efforts to simplify and improve the CFS expression of therapeutic proteins have managed to reduce the cost of the required reagents. Cai et al. (2015) developed a simplified CFS reaction mixture (master mix) and managed to provide approximately a 95% decrease in the master mix cost [9]. To capture the impact of the cost reduction related to the master mix the sensitivity analysis considered a representative range that reflects the improvements that have been made. The COG/g for an ADC would more than double if recent developments related to the master mix recipe were not taken into account. Additionally, Caschera and Noireaux (2013) demonstrated that CFS titre can reach values up to 2.3g/L by optimising metabolic pathways [34]. The analysis illustrated that doubling the CFS titre in this manner had a significant impact on the cost-competitiveness of the system with a 21% reduction in COG/g when the titre improves from 1g/L to 2g/L. Other parameters demonstrated a moderate impact on the COG/g. The conjugation yield ranked as the 3rd most impactful (13%) followed by the extract total proteins concentration (7%) and the extract volumetric concentration (5%). Finally, other key assumptions demonstrated a less significant impact on the COG/g. For instance, the duration of the CFS reaction step had no impact on the COG/g within the range that was evaluated in this study (8 – 20 hours). At a lower (25kg/y) and a higher (175kg/y) product demand, the ranking of the parameters included in the sensitivity analysis does not change from that presented in Figure 3A for an ADC demand of 100kg/y. However, the impact of these parameters is proportional to the demand as presented in the next section.

1.7. Target analysis

The analysis to reduce the COG/g included only the CFS parameters with the greatest cost-benefit on COG/g from Figure 3A and that met the threshold of achieving at least a 5% reduction in COG/g. This identified the top four cost drivers as: CFS titre (g/L), CFS conjugation yield (%), extract total protein concentration (g/L) and extract volumetric concentration (v/v). Figure 3B illustrates the potential cumulative cost-benefit that process improvements in these four parameters can have on the COG/g. Doubling the CFS titre to 2g/L offers a significant cost reduction, however, additional improvements related to the conjugation yield (from 72% to 90%) and the *E. coli* cell extract (from 20g/L to 10g/L for the total protein concentration and from 30% to 15% for the extract volumetric concentration) are necessary for the CFS process. A COG/g reduction of approximately 40% at 25kg/y and 45% at 100kg/y and 175 kg/y would be required for a CFS system to break-even with a CHO for the commercial manufacture of an ADC. The assumed process improvements managed to achieve a COG reduction of 20%, 34%, and 39% at 25kg/y, 100kg/y, and 175kg/y, respectively, and hence did not meet the targets.

The impact on the COG/g of these CFS process improvements becomes more significant as the annual product demand increases from 25kg to 175kg. For instance, by doubling the CFS titre the COG/g can be reduced by 13%, 21%, and 24% at 25kg/y, 100kg/y, and 175kg/y, respectively. Hence, the additional process development effort that could be required to improve the performance of the CFS process would have a potentially greater reward as the product demand increases.

Further improvements in the key CFS parameters beyond the best case values explored in the sensitivity analysis could reduce the COG/g to a competitive level against well-established mammalian systems. To determine the necessary improvements for a CFS system to be more competitive with a CHO system a series of contour plots were created. Figure 4 presents the change in the COG/g by varying the four parameters with the greatest COG reduction as identified through sensitivity analysis (Figure 3A). This results in a matrix of scenarios highlighting contour plots of CFS

titre and conjugation yield for different combinations of demands and extract conditions. The area on the top-right corner within the dashed border represents the operating window where the COG/g for the CFS system becomes less or equal to the CHO system. Examining the contour plots highlights the target values of the key CFS parameters to reach this area.

At the lower end of the annual product demand range that was investigated here, the CFS process could not match the COG/g value of the CHO process (Figure 4A, B). The minimum COG/g value for the CFS process at 25kg/y was estimated around 1,200$/g assuming a titre of 10g/L, a conjugation yield of 95%, an extract total protein concentration of 10g/L, and an extract volumetric concentration of 15% (Figure 4B). This minimum COG/g value for the CFS process is still 20% higher than the CHO process despite a 10-fold increase in the CFS titre. At a product demand of 100kg/y, assuming a conjugation yield of 95% can be achieved then the CFS process would need to increase its titre to a minimum of 7g/L without any improvements in the extract concentrations (Figure 4C), or 5g/L in the case where both extract concentrations are improved by 50% (Figure 4D). Finally, at a product demand of 175kg/y, assuming a conjugation yield of 95%, the minimum target for the CFS titre is 4.5g/L without any improvements in the extract concentrations (Figure 4E) or 3.5g/L in the case where both extract concentrations are decreased by 50% (Figure 4F). The area within the dashed border, where the CFS process becomes more cost-effective, increases in size as the product demand increases from 100kg/y to 175kg/y. Therefore, as the product demand rises the target values for the titre and the conjugation yield are lowered.

Furthermore, Figure 4 shows that the titre has a non-linear correlation with the COG/g. As titre increases the COG/g decreases, however, at a declining rate that can be visualised by the increasing size of the colour bands moving from left to right. Thus, increasing the CFS titre beyond a certain threshold would not have an impact on the COG/g. This trend of decreasing COG/g with increasing titre has already been demonstrated for cell-based processes for biologics [29, 35]. In this case study, the critical titre threshold was estimated around 13g/L assuming optimised conditions for the

conjugation yield (95%), extract total protein concentration (10g/L) and extract volumetric concentration (15%).

It is worth highlighting that the CHO titre of 3g/L assumed in this study is on the conservative side of the range that is routinely achieved at a commercial scale for mAbs [36]. Considering a higher titre for the CHO process would reduce its COG/g value and it would make the difference in COG/g between CFS and CHO even more pronounced. Therefore, the cost-competitiveness of CFS in large-scale manufacture is directly dependent on the progress and future improvements of other systems (e.g. CHO).

Concluding Remarks

This study evaluated the cost-effectiveness of a CFS system against a well-established mammalian cell system for the commercial manufacture of an ADC. The analysis demonstrated that further improvements in the CFS system would be necessary (i.e. increase in titre) for CFS to become more cost-competitive with a CHO platform for the commercial manufacture of therapeutic antibodies. Materials related to CFS demonstrated a major contribution to the total cost. Additionally, the manufacturing cost of the extract combined with the high quantities needed increase substantially the total COG/g. On the other hand, different process and business strategies might be considered where CFS could offer a competitive advantage due to its increased productivity given the shorter process times compared to cell-based platforms. Additionally, contract manufacturing organisations might play a significant role in the supply of cell extracts and other biological reagents at competitive prices thus potentially providing an additional level of flexibility to the CFS system.

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Conflict of interest

None declared

Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article.

Author Contribution

Christos Stamatis: Data curation-Equal, Formal analysis-Lead, Methodology-Equal, Visualization-Lead, Writing-original draft-Lead, Writing-review & editing-Equal; Suzanne Farid: Conceptualization-Lead, Data curation-Equal, Formal analysis-Supporting, Funding acquisition-Lead, Methodology-Equal, Supervision-Lead, Visualization-Supporting, Writing-original draft-Supporting, Writing-review & editing-Equal

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Table 1. List of key assumptions for the techno-economic analysis and key sizing outputs.

Key analysis inputs	Deterministic		Sensitivity
Flowsheet[(1)]	**CHO**	**CFS + Extract**	**CFS + Extract (Worst – Best)**
Extract Production			
Mode	-	Fed-batch	-
Duration	-	5 days	-
Titre (g/L)	-	14	-
Antibody Production			
Mode	Fed-batch	Batch	-
Duration	14 days	14h [9]	20 – 8
Titre (g/L)	3	1 [34]	0.5 – 2
Extract volumetric concentration (v/v)	-	0.3 [9]	0.4 – 0.2
Extract total protein concentration (g/L)	-	20 [9]	30 – 10
T7 RNA polymerase concentration (g/L)	-	0.02 [9]	0.03 – 0.01
T7 RNA polymerase activity (U/µg)	-	450 [37]	900 – 225
Available T7 RNA polymerase activity (U/µg)	-	450	-
DNA template concentration (µg/mL)	-	10 [9]	20 – 5
DNA template stock concentration (g/L)	-	1	-
Antibody Modification			
Buffer volumetric concentration (v/v)	0.2	-	-
Linker molecular weight (g/mol)	350	-	-
Linker to antibody ratio	4	-	-
Yield (%)	90 [38]	-	-
Antibody Conjugation			
Buffer volumetric concentration (v/v)	0.2	0.2	-

Cytotoxin molecular weight (g/mol)	1,000	1,350	-
Cytotoxin to antibody ratio	4	4	-
Yield (%)	80 [38]	72	50 – 90
Overall DSP yield (%)	42	45	-
Unit Costs			
CFS media component: Master mix ($/L)	-	45 [9]	650 – 30
CFS media component: T7 RNA polymerase ($/g)	-	1,000	2,000 – 500
CFS media component: DNA template ($/g)	-	1,000	2,000 – 500
CHO / *E. coli* media component: Base media ($/L)	10	10	-
CHO / *E. coli* media component: Feed media ($/L)	100	20	-
Linker ($/g)	100	-	-
Cytotoxin ($/g)	1,000	1,100	2,200 – 550
Key deterministic analysis output[(2)]	**CHO**	**CFS + Extract[(3)]**	
Number of batches	20	66 & 33	
Production bioreactor (m^3)	1; 4; 7	0.9 & 1; 3.4 & 3.8; 6 & 6.7	
Capture chromatography column (cm)	30; 60; 80	20; 30; 45	
Campaign time (days)	330	330	

Notes: The molecular weight of the monoclonal antibody was assumed 150kDa. (1) CHO refers to the CHO ADC process and CFS + Extract refers to the CFS ADC process with an in-house supply of *E. coli* cell extract, (2) when necessary outputs reported for 25; 100; 175kg/y of ADC and (3) when necessary outputs reported for CFS & Extract processes.

List of Figures

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(A)

Inputs

Product:
- Annual Demand & Target product profile

Process:
- Process flowsheet
- Number of USP & DSP trains
- Process step technology (Single-use or reusable)

Facility:
- Facility setup (i.e. Centralised, decentralised)
- Facility type (i.e. Single- or multi-product)
- Number of batches per campaign

Database

Process assumptions:
- Operating parameters (e.g. flux, resin capacity)
- Performance (e.g. titre, yield, impurity removal)
- Resources requirements

Facility assumptions:
- Operational time
- Shift patterns
- QC requirements

Resources:
- Equipment (reference sizes, footprint, unit cost)
- Consumables (reference sizes, unit cost)
- Reagents (unit cost)

Economic data:
- Cost factors (e.g. supervisors, general utilities)
- Footprint coefficients

Techno-economic engine: *Therapeutic proteins*

Technical calculations:
- Mass balance
- Sizing of equipment & consumables
- Consumption of reagents & consumables
- Equipment & facility footprint
- Batch & campaign scheduling
- Number of operators required

Economic calculations:
- Equipment cost
- Raw materials cost
- Labour cost
- Capital investment
- Cost of goods

Outputs

Key financial metrics: COG/g & FCI

Product: Product & impurities profile

Process:
- Batch duration & schedule
- Equipment size & utilisation
- Resources consumption

Facility:
- Footprint
- Campaign time & utilisation

Capital investment breakdown by stage

Cost of goods breakdown:
- By category (materials, labour, facility-related)
- By process step

(B)

	CHO ADC process	CFS ADC process	*E. coli* cell extract process
mAb process steps	Inoculum Grow-up & Seed Train	-	Inoculum Grow-up & Seed Train
	Production Bioreactor	Production Bioreactor ←	Fermentation
	Centrifugation (85%)	Centrifugation (85%)	Centrifugation (95%)
	Depth Filtration (95%)	Depth Filtration (95%)	Homogenisation (100%)
	Capture Chromatography (97%)	Capture Chromatography (97%)	Centrifugation (85%)
	Virus Inactivation (95%)	-	Depth Filtration (95%)
	Intermediate Chromatography (95%)	Intermediate Chromatography (95%)	Capture Chromatography (97%)
	1st Polishing Chromatography (97%)	1st Polishing Chromatography (97%)	-
	Virus Filtration (98%)	-	Intermediate Chromatography (95%)
	Concentration & Buffer Exchange (95%)	Concentration & Buffer Exchange (95%)	1st Polishing Chromatography (97%)
Conjugation process steps	Antibody Modification (90%)	-	-
	Antibody Conjugation (80%)	Antibody Conjugation (72%)	Concentration & Buffer Exchange (95%)
	2nd Polishing Chromatography (95%)	2nd Polishing Chromatography (95%)	-
	Formulation (95%)	Formulation (95%)	Antibody Conjugation (72%)
			2nd Polishing Chromatography (95%)
			Formulation (95%)

Figure 2: (A) COG/g breakdown by category for the manufacture of an ADC using a CHO and a CFS process with in-house production of E. coli cell extract at an annual product demand of 100kg/y. (B) Breakdown of the cost of materials among the main stages of the process at 100kg/y. (C) Breakdown of the cost of materials among unit operations.

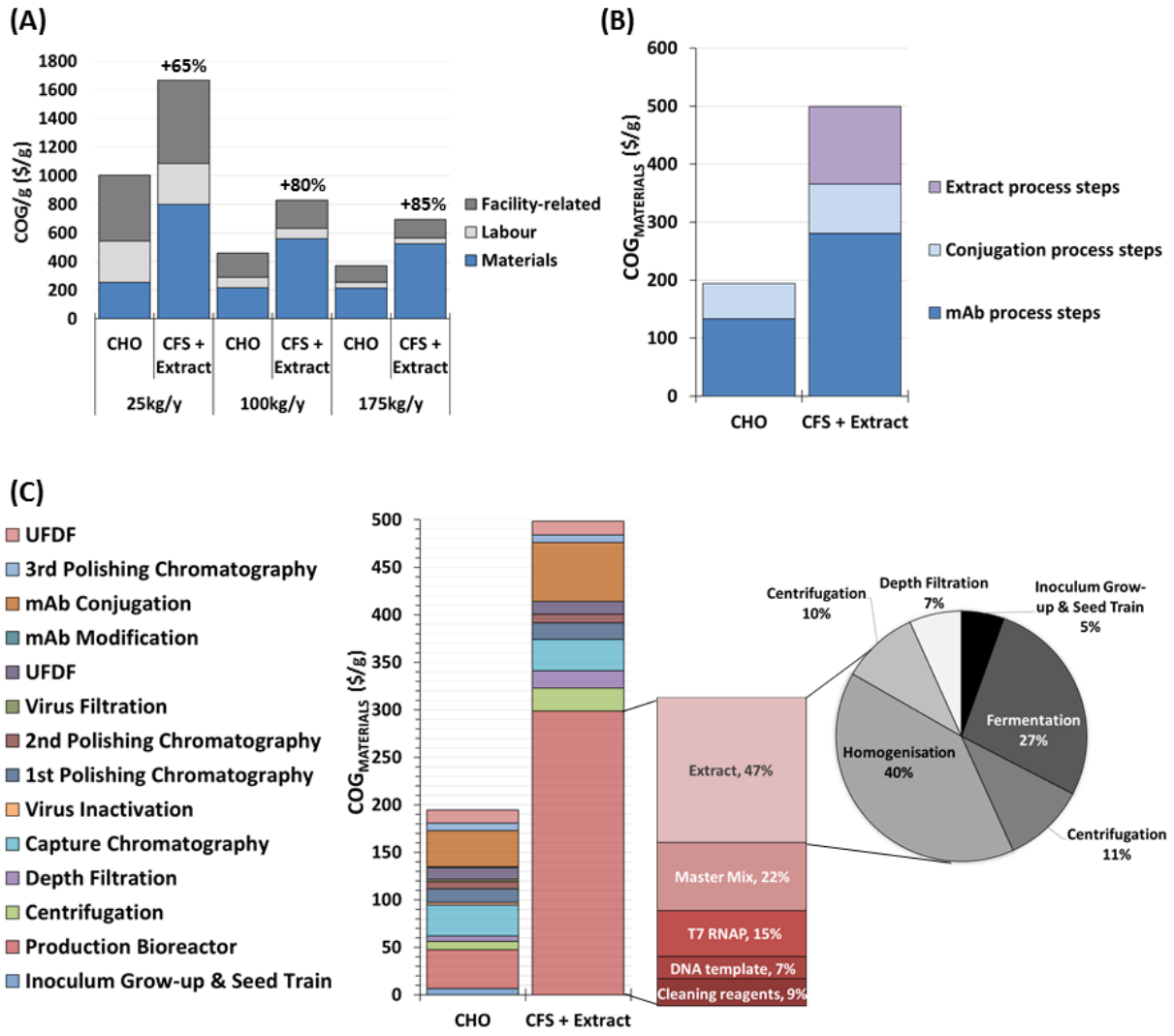


Figure 3: (A) Tornado graph visualising the impact of key model assumptions on the COG/g for the CFS system at 100kg/y. (B) Illustration of the cumulative impact of the improvements to the CFS process on the COG/g across a range of annual product demands and break-even point with a CHO process.

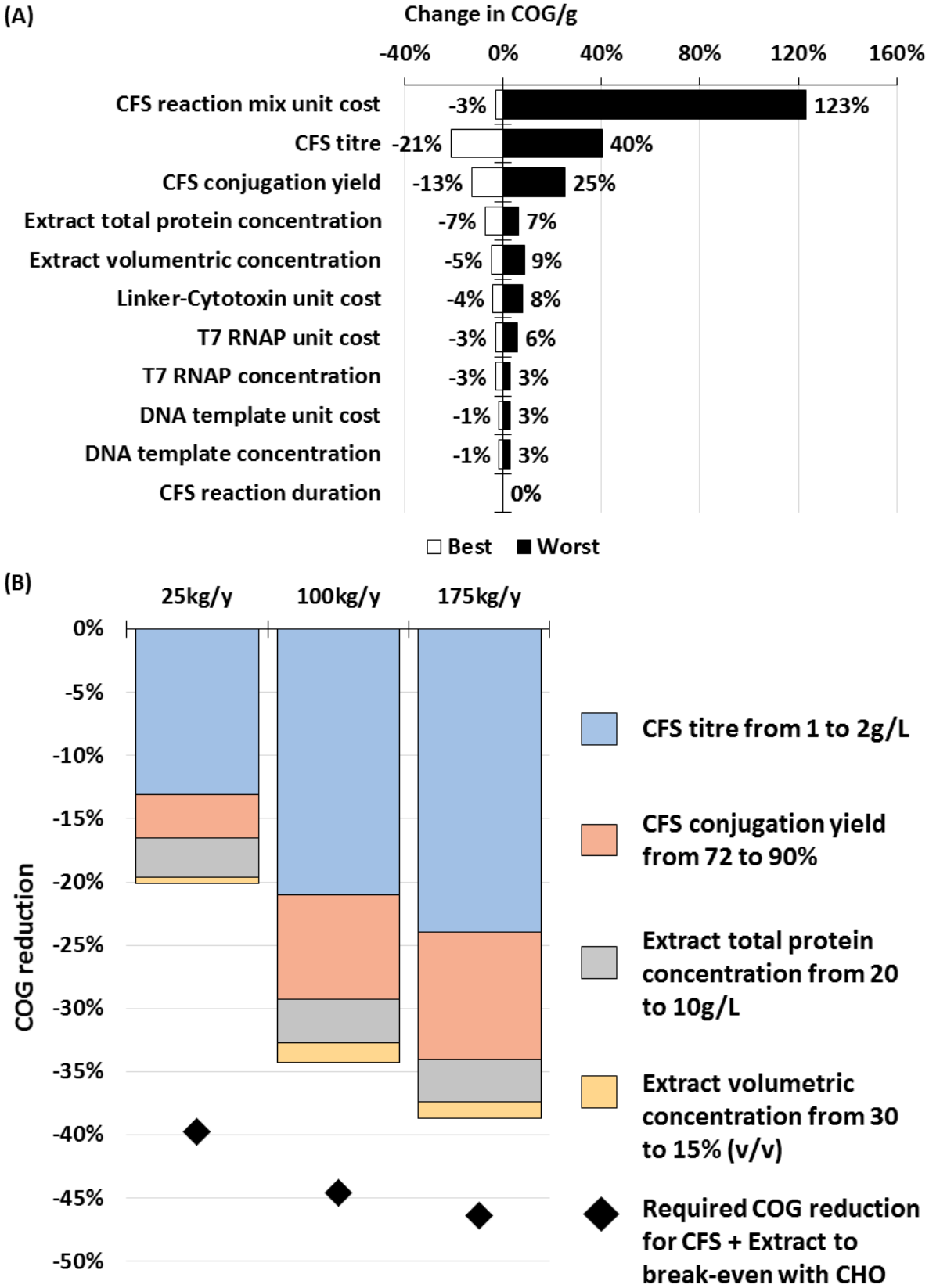
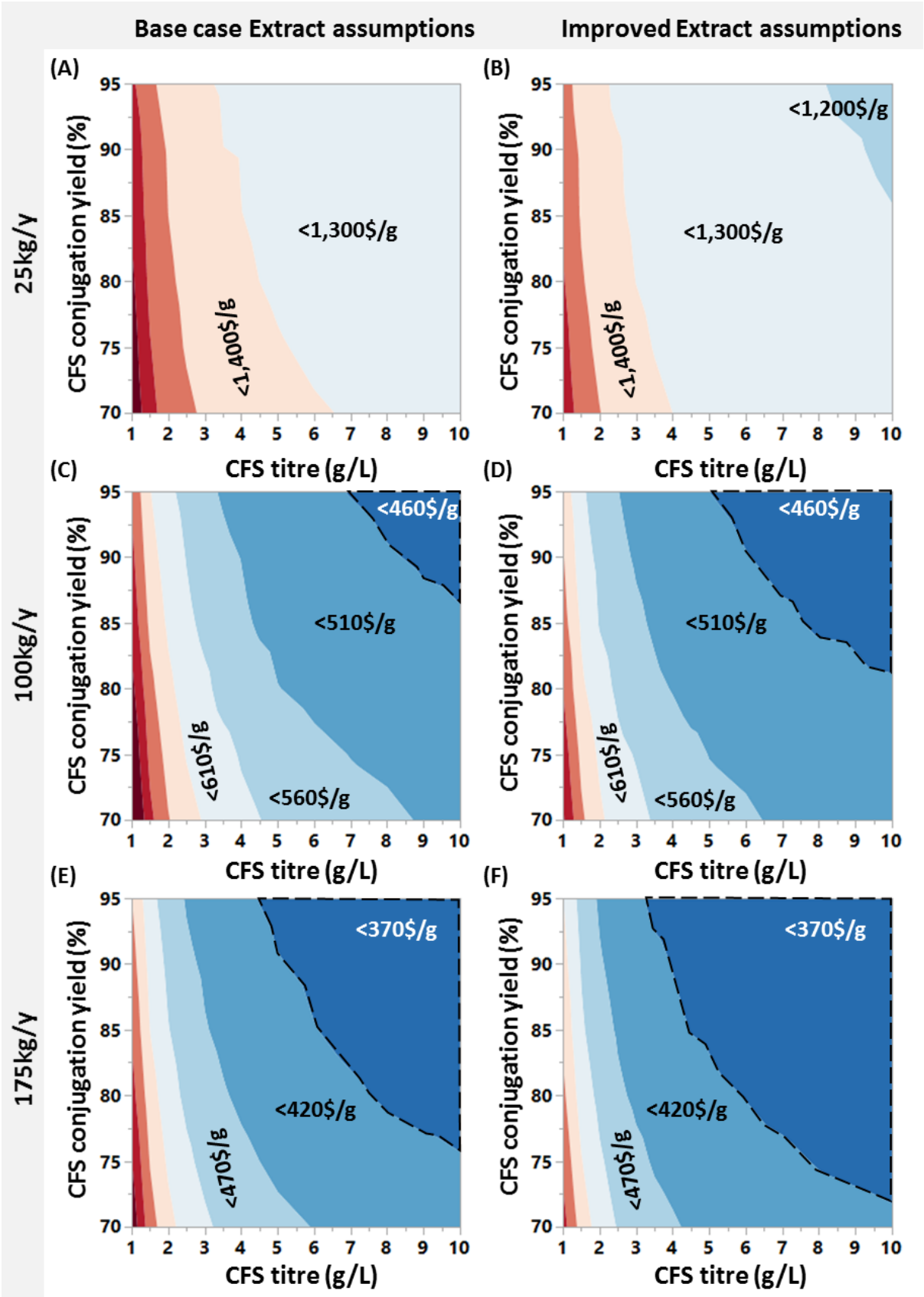
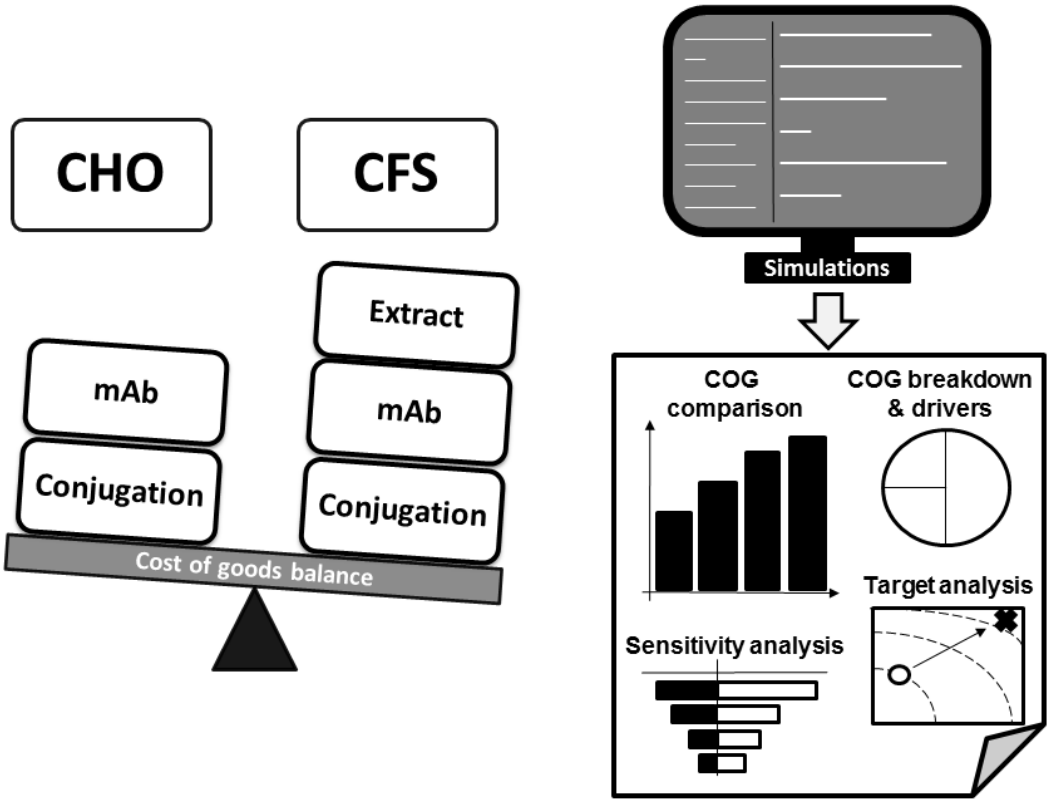


Figure 4: Contour plots to determine the operating window where a CFS process with an in-house supply of E. coli cell extract becomes more cost-competitive to a CHO process. (A) and (B) are for an ADC demand of 25kg/y, (C) and (D) are for an ADC demand of 100kg/y, and (E) and (F) are for an ADC demand of 175kg/y. Also (A), (C) and (E) consider an extract total protein concentration of 20g/L and an extract volumetric concentration of 30% (base case extract), while (B), (D) and (F) consider an extract total protein concentration of 10g/L and volumetric concentration of 15% (improved extract).



Graphical abstract



Cell-free synthesis (CFS) has demonstrated significant improvements over the past five decades with the biopharmaceutical industry evaluating and developing the technology for the commercial manufacture of biologics. In this study, the authors performed a process economics analysis to evaluate CFS for the commercial manufacture of an antibody-drug conjugate. The analysis demonstrated that currently CFS shows approximately 80% higher cost of goods and that further process improvements are necessary for CFS to become more cost-competitive.