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

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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 a range of commercial scenarios. 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 antibody drug conjugate (ADC) at different commercial product demand levels (100 – 1000kg/year). The analysis demonstrated the potential of CFS for the commercial manufacture of biologics and identified key cost drivers related with the system. The CFS system showed approximately a two-fold 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 impetus for further process improvements especially in the titre for the CFS process to become more competitive against well-established systems.

Keywords

Cell-free synthesis, process economics, techno-economic analysis, biologics, manufacture

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 long production cycles that typically span 2-3 weeks. Cell-free synthesis (CFS) has emerged as an alternative to cell-based systems for the commercial production of biologics within hours instead of weeks. 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) in order to gain an understanding of the process economics of the system compared to a cell-based platform.

Typically, commercial manufacture of therapeutic proteins is achieved using living cells taking advantage of cell proliferation and of the intracellular machinery for the expression of the desired product. Using a living cells system usually requires extensive cell engineering and development effort to program the cells in delivering the protein of interest (Costa et al., 2010). Therefore, cell-based methods are commonly associated with long development and manufacturing timeframes (Zhu, 2012).

CFS is a system that combines a number of chemical and biological reagents in order to mimic in vitro specific biological functions (Carlson et al., 2012; Hodgman and Jewett, 2012; Lu, 2017). Thus, due to the absence of a cell wall, monitoring and control of the process can be significantly improved. Published work has evaluated the CFS system for the production of monoclonal antibodies (Cai et al., 2015), bispecific antibodies (Xu et al., 2015), antibody drug conjugates (Zimmerman et al., 2014; Kline et al., 2015), fusion proteins (Kanter et al., 2007), peptides (Murray and Baliga, 2013) and vaccines (Kanter et al., 2007). 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 monoclonal antibodies (Cai et al., 2015). Moreover, recent advancements such as a simpler reaction mixture (Cai et al., 2015), reproducible protocols for the production of bacterial (Yang et al., 2012; Katsura et al., 2017) and mammalian cell extracts (Brodel, Sonnabend, and Kubick, 2013) and demonstration of scalability (Voloshin and Swartz, 2005; Zawada et al., 2011), combined with the potential to accelerate drug development and manufacture timelines (Ogonah et al., 2017), have triggered interest in the potential of CFS for the commercial manufacture of biologics.

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 long history in developing decision-support tools to address challenges in bioprocessing from the process (e.g. Simaria et al., 2012) and the facility (e.g. Stonier et al., 2012) to the portfolio level (George & Farid, 2008). 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 COG/g of whole bioprocesses based on CFS versus a mammalian cell-based system (CHO) for the commercial manufacture of an ADC. In-depth analysis of the 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

Model structure

An object-oriented simulation engine was developed in Python^{TM} (v3.6), edited in Spyder (v3.2.6) and operated through Jupyter Notebook (v5.2.2). The simulation engine follows a hierarchical structure from the definition of a therapeutic protein to the design of the required process flowsheets in a bio-manufacturing facility. Design calculations for different unit operations were based upon previous work conducted at UCL Biochemical Engineering (Farid, 2001; Farid et al., 2007; Stonier et al., 2012; Simaria et al., 2012; Pollock et al., 2013). Fixed capital investment and cost of goods metrics were determined as outlined in Farid (2001) and Farid et al. (2007). Furthermore, new design calculations were introduced to simulate the CFS reaction. Given the required working volume for the production bioreactor, the required volume of each main components in the CFS reaction was estimated as follows:

$$V_{\text{Extract}} = V_{\text{Bioreactor working volume}} * \nu_{\text{Extract}}$$

$$V_{T7RNAP} = \text{SA}_{T7RNAP} * C_{T7RNAP} * V_{\text{Bioreactor working volume}}/\text{AA}_{T7RNAP}$$

$$V_{\text{DNA}} = C_{\text{DNA}} * V_{\text{Bioreactor working volume}}/\text{SC}_{\text{DNA}}$$

$$V_{\text{Master mix}} = V_{\text{Bioreactor working volume}} - V_{\text{Extract}} - V_{T7RNAP} - V_{\text{DNA}}$$

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 database containing default values for key assumptions for each unit operation and costs for different process equipment and materials. 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 in a different MS Excel worksheet for further analysis and visualisation. Figure 1a illustrates a schematic of the structure of the techno-economic engine and its main components.

Scenario analysis formulation

To evaluate the cost-effectiveness of a CFS system, a case study was formulated comparing its COG/g against a CHO system for the commercial manufacture of an ADC. Table I summarises the key assumptions used by the techno-economic engine for the purpose of the case study. It should be highlighted that the weighted average media price is not a model parameter but rather a calculated value that indicates the difference in the cost of reagents among CHO, CFS and *Escherichia coli*(*E. coli*) processes. In the case of CHO and *E. coli* the model considers two media recipes; one for the batch phase and one for the fed-batch phase of the production. On the other hand, for the CFS system there are four main reaction components; cell extract, master mix, T7 RNA polymerase and DNA template.

A range of annual product demands 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 were 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.

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 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. 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-cytotoxin molecule (Zimmerman et al., 2014). Finally, due to the absence of a mammalian cell line in the CFS process, the virus inactivation and nanofiltration steps have been removed. Finally, the manufacturing costs were estimated for the drug substance and not for the drug product thus excluding the final fill-finish 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. The minimum number of CFS batches that a single *E. coli* batch could supply enough material, depends on the delivery time for each process flowsheet. In case of a single batch, the delivery time is simply the duration of the batch. However, in case of multiple batches the delivery time, after the first batch, could be reduced due to the overlap of an upstream processing (USP) train with the downstream processing (DSP) train of the previous batch. That overlap is a function of the USP and DSP duration and the number of bioreactors operated in staggered mode. In order to perform a fair comparison between CHO and CFS, the number of batches and the number of staggered production bioreactors for each process flowsheet were optimised using a brute force algorithm

searching for the minimum COG/g. Two constraints were considered to ensure the campaign time is less than 340 days and to synchronise the *E. coli* extract process with the ADC process.

For the economic evaluation of the CFS system through the scenario 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^{TM} and Jupyter Notebook) using the worst and best case values for each assumption in Table II.

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 them 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.

Scale and facility utilisation

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 cost of goods per gram of product (COG/g) was used as a comparator. Additionally, the utilisation of the facility for the CHO and the CFS process was optimised using a brute force algorithm to minimise the COG/g for each system and allow the possibility of introducing multiple products into the facility.

A summary of the key sizing and operational results is provided in Table III. 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 the model predicted that the optimal facility utilisation (within the operating weeks) was above 88% throughout the demands. On the other hand, with the CFS process the optimal facility utilisation increased from 55% to 75% and 90% as the demand increased from 100 to 500 and 1000kg/year. Hence, the CFS process has the potential to provide more operational flexibility to the facility, particularly for lower demands where the use of CFS frees up facility time to be used for other products in the pipeline.

The speed of a CFS process allows for a greater number of batches that would typically translate into a smaller bioreactor size if titres were equivalent. However, the lower titre of the CFS reaction compared to the CHO cell culture limits any potential benefits in process equipment sizing. For instance, to achieve a target demand of 100kg/year, the optimal number of batches for the CHO and CFS systems were 20 and 30 batches with a campaign duration of 334 and 181 days and a bioreactor working volume of 4,000L and 7,500L, respectively. However, at larger demands of 500kg and 1000kg/year the CHO process requires the use of three bioreactors in staggered mode to minimise the COG/g compared to a single bioreactor for the CFS process. Therefore, although the working volume of a single bioreactor is lower for the CHO process compared to the CFS process, the total required capacity is higher for the CHO process.

Cost of goods breakdown

A breakdown of the COG/g for each system and across different annual product demands is presented in Figure 2a. The breakdown in Figure 2a categorises costs as labour, materials and indirect. Labour costs include the cost of operators, supervisors, management and quality control and assurance personnel (Farid, 2001; Farid et al., 2007; Simaria et al., 2012). The cost of materials accounts for reagents (media, buffers, cleaning agents, etc.), consumables (filters and resins) and miscellaneous materials. Indirect costs consider the depreciation of the fixed equipment, the maintenance and insurance of the facility along with general

utilities (e.g. HVAC) and local taxes (Farid, 2001). It should be noted that the costs (labour, materials and indirect) in Figure 2a 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 2 – 2.25 fold higher compared to a CHO system for the manufacture of an ADC throughout different product demands (Figure 2a). This significant cost increase can be mainly attributed to the in-house manufacture of the *E. coli* cell extract. The values of COG/g of ADC attributed to the*E. coli* process range from 275$/g to 215$/g at 100kg/year to 1000kg/year scales, which represents approximately 40% to 30% of the COG/g, respectively. Additionally, the unit cost of the in-house manufacture of the *E. coli* cell extract decreases from 20$/g to 16$/g as the demand of ADC increases from 100kg/year to 1000kg/year. 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 in order 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 in order to determine the key improvements needed.

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). So the next stage was to determine which process stages and unit operations were contributing the most. Focusing on the cost of materials, Figure 2b presents a breakdown among the main stages of the process demonstrating the portion of the cost of materials due to the in-house manufacture of the *E. coli* extract. Almost a third 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 3 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/year. The percentage cost of materials for the production bioreactor (cell-free protein synthesis step) increases from 29% for the CHO process to 62% 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 56%. The other CFS reaction components (master mix, T7 RNA polymerase and DNA template) account for 33% with the remaining cost attributed to cleaning reagents (11%) and consumables (0.1%). Finally, the pie chart in Figure 3 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 more than 70%.

The cost breakdowns in Figure 3 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 in order to determine the focus of further process development and reduce the COG/g. This is explored in the next sections.

Sensitivity analysis

The deterministic analysis of a CFS system was based upon certain assumptions regarding its operation and associated costs (Table I). 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 II). Figure 4 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 evidently 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. 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 the CFS titre can reach values up to 2.3g/L by optimising metabolic pathways. The tool illustrated that doubling the CFS titre in this manner had a significant impact on the cost-competitiveness of the system with a ˜30% reduction in COG/g when the titre improves from 1g/L to 2g/L. 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).

Target analysis

The analysis to reduce the COG/g considered only the CFS parameters with the greatest cost benefit on COG/g from Figure 4: CFS titre (g/L), CFS conjugation yield (%), extract total protein concentration (g/L) and extract volumetric concentration (v/v). Figure 5 illustrates the potential cumulative cost benefit that process improvements can have on the COG/g. Evidently, 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 30% to 20% for the extract volumetric concentration and from 20g/L to 10g/L for the total protein concentration) are necessary for the CFS process to offer similar COG/g with the CHO process. A COG/g reduction of 50% at 100kg/year and 55% at 500 and 1000 kg/year would be required for a CFS system to break-even with a CHO for the commercial manufacture of an ADC. The assumed process improvements manage to achieve a 46%, 52% and 53% cost reduction at 100, 500 and 1000kg/year, respectively, demonstrating similar but still higher COG/g values compared to the CHO process.

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. Focusing on the case of 100kg/year, Figure 6 presents the change in the COG/g by varying four parameters. For the top and bottom plots an extract total protein concentration of 20g/L and 10g/L, respectively, is assumed. Additionally, for the right and left plots an extract volumetric concentration of 30% and 20%, respectively, is assumed. Finally, each plot has the CFS titre on the X-axis and the CFS conjugation yield on the Y-axis.

Using the base and best case values for these assumptions from Table II, the COG/g values for the CFS process are indicated (with the arrows) on the top-left plot and the bottom-right plot of Figure 6, respectively. The darkest coloured area on the top-right corner within the dashed border of each plot represents the space where the COG/g for the CFS system becomes less or equal to the CHO system. Examining the contour plots highlights target values of the key CFS parameters to reach this zone. On the top-left plot where no improvements to extract conditions are assumed, the titre needs to increase 4 – 5 fold in combination with the conjugation yield increasing from 72% to 85 – 95%. Alternatively, focusing on the bottom-right plot with the optimised extract conditions, slightly lower target improvements in titre and yield are required. In this case, the CFS titre would need to become at least equal to the CHO titre (at 3g/L) along with the conjugation yield increasing to 75 – 95% for the COG/g to achieve similar values (around 400$/g). Similar CFS titre targets were found at 500kg/year and 1000kg/year to break-even with the CHO process.

Furthermore, Figure 6 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 have a minimum impact on the COG/g. This trend of decreasing COG/g with increasing titre has already been demonstrated for cell-based processes for biologics (Farid, 2009; Stonier et al., 2012). This critical titre threshold is approximately 9-10g/L across 100-1000 kg/year. These values were derived assuming optimised conditions for the conjugation yield (95%), extract volumetric concentration (20%) and extract total protein concentration (10g/L).

It is worth noting that the CHO titre of 3g/L assumed in this study is on the conservative side of the range

that is routinely achieved at commercial scale for monoclonal antibodies. Therefore, it should be highlighted that the cost-competitiveness of CFS in large scale manufacture is directly dependant on the progress and improvements of other systems (e.g. CHO).

Conclusions

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) in order 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 for 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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Table I. Key assumptions for the scenario analysis specified in the user-interface of the techno-economic engine

Assumption	**Flowsheet***	**Flowsheet***
	CHO	**CFS + Extract**
Extract Production		
Duration	-	5 days
Titre (g/L)	-	14
Antibody Production		
Duration	14 days	14 hours
Titre (g/L)	3	1
Extract volumetric concentration (v/v)	-	0.3
Extract total protein concentration (g/L)	-	20
T7 RNA polymerase concentration (g/L)	-	0.02
T7 RNA polymerase activity (U/mg)	-	450,000
Available T7 RNA polymerase activity (U/mg)	-	450,000
DNA template concentration (μg/mL)	-	10
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	-

Assumption	Flowsheet*	Flowsheet*
Yield (%)	90	
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	72
Unit Costs		
CFS media component: Master mix ($/L)	-	45
CFS media component: T7 RNA polymerase ($/g)	-	1,000
CFS media component: DNA template ($/g)	-	1,000
CHO / *E. coli* media component: Growth media ($/L)	10	-
CHO media component: Expression media ($/L)	100	-
E. coli media component: Expression media ($/L)	75	
Linker ($/g)	100	-
Cytotoxin ($/g)	1,000	1,100

Note: *CHO refers to CHO ADC process, CFS refers to CFS ADC process and*E. coli* to *E. coli* cell extract process.

Table II. Key assumptions used in the sensitivity analysis to identify parameters with significant impact on COG/g for the cell-free synthesis system

Assumption	Worst	Base	Best	Reference for Base Value
CFS reaction duration (hr)	20	14	8	Cai et al., 2015
CFS titre (g/L)	0.5	1	2	Caschera and Noireaux, 2013; Cai et al., 2015
Extract volumetric concentration (v/v)	0.4	0.3	0.2	Cai et al., 2015
Extract total protein concentration (g/L)	30	20	10	Zawada et al., 2011
T7 RNA polymerase concentration (g/L)	0.03	0.02	0.01	Cai et al., 2015
DNA template concentration (µg/mL)	20	10	5	Cai et al., 2015
T7 RNA polymerase specific activity (U/mg)	225,000	450,000	900,000	Sampson and Uhlenbeck, 1988; Li, Wang and Wang, 1999

Assumption	Worst	Base	Best	Reference for Base Value
CFS conjugation yield (%)	50	72	90	Beck et al., 2017
Master mix unit cost ($/L)	650	45	30	Cai et al., 2015
T7 RNA polymerase unit cost ($/g)	2,000	1,000	500	*Assumed*
Linker-Cytotoxin unit cost ($/g)	2,200	1,100	550	*Industrial experts*
DNA template unit cost ($/g)	2,000	1,000	500	*Assumed*

Table III. Summary of key scale and operational outputs

Demand (kg/year)	Flowsheet[1]	Number of batches	Total bioreactor capacity (m^3)[2]	Capture chromato
100	**CHO**	20	1 x 4	60
	CFS	30	1 x 7.5	45
	Extract	15	1 x 8.5	-
500	**CHO**	50	3 x 8	80
	CFS	50	1 x 22.5	80
	Extract	25	1 x 25	-
1000	**CHO**	50	3 x 16	110
	CFS	60	1 x 37.5	100
	Extract	30	1 x 42	-

Notes: 1: CHO refers to CHO ADC process, CFS refers to CFS ADC process and *E. coli* to *E. coli* cell extract process, 2: number of bioreactors in staggered mode x bioreactor working volume

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Figure 1. a) Schematic illustration of the structure of a techno-economic engine for biologics. b) Process flowsheets for the manufacture of an ADC using a CHO and a CFS process with an in-house supply of *E. coli* cell extract.

Figure 2. a) COG/g breakdown for the manufacture of an ADC using a CHO and a CFS process with in-house production of *E. coli* cell extract at different annual product demands. b) Breakdown of the cost of materials among the main stages of the process at 100kg/year.

Figure 3. Breakdown of the cost of materials among unit operations at 100kg/year of ADC.

Figure 4. Tornado graph visualising the impact of key model assumptions on the COG/g for the CFS system at 100kg/year.

Figure 5. Visualisation of the cumulative impact of the improvements to the CFS process on the COG/g at different annual product demands and break-even points with a CHO process.

Figure 6. Contour plots to determine the operating space where a CFS process with in-house supply of *E. coli* cell extract becomes more cost-competitive to a CHO process for the manufacture of an ADC at

100kg/year.