Decision-support tool for bioprocess economics and sustainability analysis of end-to-end continuous antibody manufacturing strategies

Thesis submitted to University College London for the degree of Doctor of Philosophy (PhD) in Biochemical Engineering

By

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I, Catarina Pereira Galo Neves, confirm that the work presented in my thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

To my mother.

Abstract

The biopharmaceutical industry is navigating a dynamic landscape marked by heightened competition, cost pressures, and the pursuit of innovative manufacturing solutions. As a result, the sector is exploring new manufacturing avenues in continuous mode, with renewed interest in the potential of column-free capture alternatives for monoclonal antibody (mAb) production. This stems from a desire to reduce manufacturing costs and to align with global commitments to achieve net zero carbon emissions. In addition, concerted efforts are being directed to automate the control of continuous bioprocesses to enhance quality control and process performance levels. This thesis aims to create a decisional tool that facilitates an integrated evaluation of the economic and environmental aspects of end-to-end continuous antibody manufacturing routes, with a focus on column-free routes and automated control.

A comprehensive framework for modelling the economic, environmental, and technological dimensions of end-to-end continuous manufacture was developed. The trade-offs of integrating the column-free options of precipitation or aqueous two-phase extraction in mAb capture on end-to-end continuous flowsheets were quantified with a process economics model. The assessment incorporated deterministic analysis, Monte Carlo simulations and multi-criteria decision making techniques and showed that continuous manufacturing was preferable over batch and that column-free based flowsheets could offer economic advantages for processes with intensified cell culture productivities and optimised yields.

On the environmental front, a life cycle assessment of different manufacturing alternatives demonstrated that the key drivers of product carbon footprint were related to energy use and material supply. For batch processes, emissions were mostly related to a high energy consumption related to larger facilities, while for continuous processes the carbon footprint from reagents and consumables fabrication was a key driver. Carbon reduction strategies were identified and the flowsheet with product precipitation showed the most accentuated decrease in carbon emissions after process optimisation.

Finally, the current state-of-the-art and vision for the implementation of process analytical technologies (PAT) in bioprocessing were investigated by conducting

a survey and a series of interviews with global industrial and academic experts. The simulation tool also demonstrated the potential impact of PAT to decrease manufacturing costs, with a payback time of less than one year on the PAT investment.

The work in this thesis showcased the added value of a simulation framework that provides an in-depth evaluation of different technologies, flowsheets and scenarios and streamlines the route to industrialisation for end-to-end continuous manufacture.

Impact statement

Decisional tools have been empowering the biopharmaceutical sector in identifying cost-saving opportunities and efficient manufacturing approaches. By leveraging computational models, these tools allow for the integration and evaluation of multiple scenarios that can be adjusted to each company's manufacturing schemes and products, enabling more informed decision-making and, ultimately, accelerating the development or improvement of life-saving therapeutics. The research outlined in this thesis offered a novel framework that integrated not only the cost assessment of end-to-end continuous strategies, but also a comprehensive environmental analysis of said schemes. As companies have been clearly stating their net zero ambitions and working towards more economic and environmentally sustainable processes, the tools developed in this work provide tangible benchmarks that are highly useful for the sector. The proposed framework can also identify early on the combination of technical parameters, both from upstream or downstream processing, that can be used to minimise cost of goods or carbon footprint. Additionally, this work united the economic and environmental assessment of continuous manufacture with the prospect of mAb continuous facilities of the future, with enhanced control and more automated systems, which aligns well with the Industry 4.0 vision. With these tools, companies can explore which process analytical technologies are the most attractive for their processes and gain insights into the level of investment they would have to commit to benefit from enhanced control in their facilities.

Industrial experts who were interviewed for specific topics of this study underscored the practical significance of the present research, as it provides decision-makers with the tools and insights needed to navigate complex process development ideas. Also, partners from academia endorsed this thesis's potential impact as it delivers relevant knowledge for future academic research endeavours.

Acknowledgments

First and foremost, I would like to acknowledge the crucial role of Prof. Suzy Farid as my supervisor. I am very grateful for having started this project under her guidance, not only due to the insightful discussions, diligent revisions or openness towards new ideas but especially due to her patience and support during difficult periods in these last years.

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On a personal note, I would like to share my gratitude to all my friends, whether nearby or far away, who contributed to maintaining my motivation up in times spirits were down. To Jose Quirino, thanks for the endless patience and for making me believe that this thesis was possible.

Lastly, my warmest words of appreciation are addressed to my mother, Helena Neves, whose love and care held me together in face of all adversities.

Table of contents

List of abbreviations

AEX	Anion-exchange chromatography
ATF	Alternating tangential filtration
ATPE	Aqueous two-phase extraction
BCF	Blood clotting factor
BHK	Baby hamster kidney
CAC	Continuous annular chromatography
CAPEX	Capital Expenditure
CAR-T	Chimeric antigen receptor T
CEX	Cation-exchange chromatography
CHO	Chinese hamster ovary
CIP	Clean-in-place
CM	Contract manufacturing
CMO	Contract manufacturing organisation
COG	Cost of goods
CPP	Critical process parameters
CQA	Critical quality attributes
DBC	Dynamic binding capacity
DSP	Downstream processing
EPO	Erythropoietin
FCI	Fixed Capital Investment
FDA	Food and Drug Administration
G-CSF	Granulocyte-colony stimulating factor
GHG	Greenhouse gas emissions
GMP	Good manufacturing practices
HCCF	Harvested cell culture fluid
HEK	Human embryonic kidney
HVAC	Heat, ventilation and air conditioning
ICB	Integrated continuous bioprocessing
IEX	Ion-exchange chromatography
IgG	Immunoglobulin G
ILC	Inline concentration

ILD	Inline dilution
IND	Investigational new drug
ItLC	Iterative learning controller
LC	Liquid chromatography
LCA	Life cycle assessment
mAb	Monoclonal antibody
MCDM	Multi-criteria decision-making
MCSGP	Multi-column solvent gradient chromatography
MILP	Mixed-integer linear programming
MINLP	Mixed-integer non-linear programming
MVDA	Multi-variate data analysis
NPV	Net present value
OPEX	Operating expenses
PAT	Process analytical technologies
QCQA	Quality Control & Quality Assurance

List of figures

List of tables

Chapter 1: Scope and Background

1.1 Introduction

The biopharmaceutical sector has demonstrated sustained progress in addressing the gaps that would enable a shift from batch to integrated continuous bioprocesses, driven by the desire to increase productivity and flexibility while reducing costs (e.g., Konstantinov and Cooney, 2015; Schofield, 2018; Mahal, Branton and Farid, 2021; Rathore, Thakur and Kateja, 2023). Moreover, with the increasing focus on achieving net zero emissions, there is a growing emphasis on adopting sustainable and eco-friendly practices (BioPhorum, 2023), and continuous manufacturing has the potential to act as an enabler for smaller facility footprints that may facilitate achieving these targets. Notable investments in continuous processing plants by both biopharma companies (e.g., Sanofi, Framingham, MA) and contract development and manufacturing organisations (e.g., Fujifilm Diosynth Biotechnologies, Billingham, UK) (Stanton, 2019a & 2019b) have been underscoring the industry's commitment to this transition. Additionally, given that purification steps constitute a significant portion of bioprocess costs, there is a trend towards exploring alternative configurations and unit operations to enable more cost-effective separations. One such area of interest is the exploration of column-free alternatives to protein A (ProA) chromatography for monoclonal antibody (mAb) capture. Despite this renewed interest, there remains a lack of a definitive business case for implementing such techniques at large scale.

The aim of this thesis is to develop and apply a decisional tool that enables a comprehensive evaluation of column-based and column-free capture steps in mAb manufacture and to shed light on the economic and environmental feasibility of such production schemes in conjunction with enhanced process control. The investigation of end-to-end continuous manufacturing will be based on seamless and uninterrupted schemes from cell culture to drug substance.

This introductory chapter aims to provide insight into the potential of biopharmaceuticals in the medicines market (**Section 1.2** and **Section 1.3**) and the inherent complexities of biomanufacturing that are driving the efforts for

innovation at clinical and commercial scales of biologics production (**Section 1.4**). **Section 1.5** provides background on several technologies used in biologics manufacture and explores the rising trend in running in continuous mode. A range of decision-support tools, including simulation software, is also reviewed in **Section 1.6**.

The aims and structure of the present thesis are described in **Section 1.7**.

1.2 The rise and evolution of biopharmaceuticals

Genetic engineering enabled the cloning of human insulin genes in *Escherichia coli* and the commercialisation of insulin by Genentech and Eli Lilly; this development made insulin the first biomolecule with pharmaceutical properties that was approved by the FDA in 1982. Three years later, two other products, human growth hormone and tissue-plasminogen activator, were also approved and introduced by Genentech to treat children with growth hormone deficiency and resolve blood clots in patients with acute myocardial infarction, respectively (Nielsen, 2013).

The term "biopharmaceuticals" was coined during the 1980s to describe pharmaceutical molecules produced through biotechnological processes and molecular biology techniques. Today, biopharmaceuticals stand as a remarkable accomplishment of modern science, addressing the limitations of synthetic drugs and un-met medical needs and offering heightened activity, specificity, and a reduced likelihood of causing side effects during treatment (Wilson and Neumann, 2012; Kesik-Brodacka, 2018). The soaring demand for biopharmaceuticals has driven substantial profits, prompting major pharmaceutical corporations to shift their research and production focus toward large-molecule products.

Figure 1.1 exhibits the sale forecast increase of biologics for 2027 and confirms that biologics have cemented their position, slightly surpassing small molecules and emerging as the primary drivers of value generation for major pharmaceutical companies. The production scale for the different modalities presented in the distribution shown in **Figure 1.1** was not available. However,

it can be assumed that small molecules are produced in larger quantities, while biologics typically command higher prices.

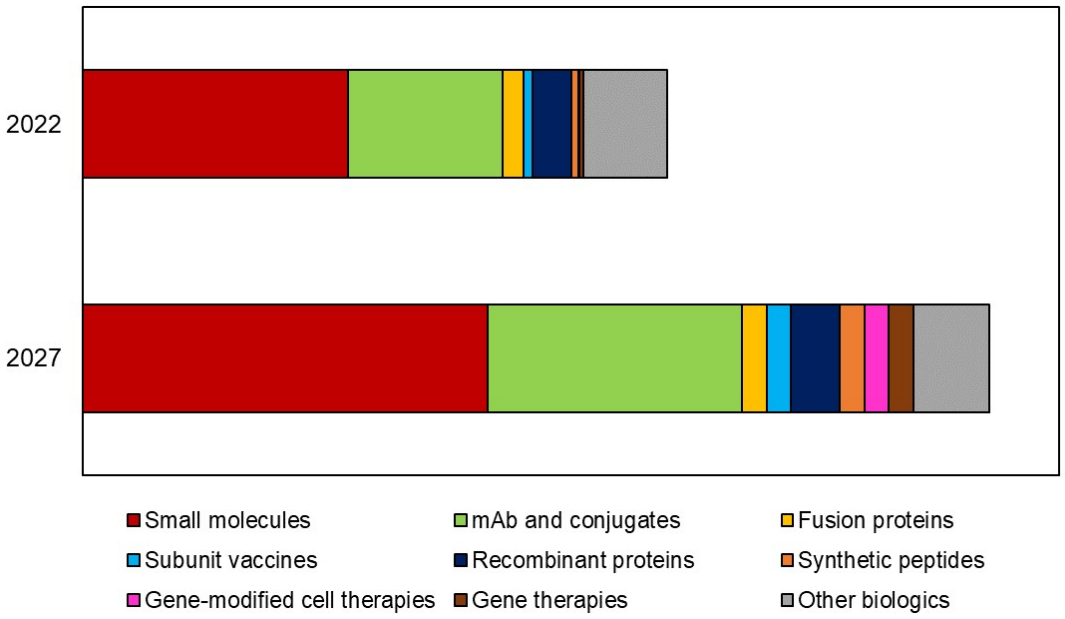

Figure 1.1 - Forecast sales ($) distribution of biologics versus small molecules by 2027. Sourced from (GlobalData Healthcare, 2022).

The remainder of this chapter provides insights into the processes involved in biopharmaceutical drug development, the applications of biopharmaceuticals, and the challenges the industry faces as market competition intensifies.

1.3 Applications and Market Landscape of Biopharmaceuticals

The escalating elderly population afflicted by chronic conditions like diabetes, cancer, and autoimmune disorders is a pivotal driver behind the continuous growth of the biopharmaceutical market (Kesik-Brodacka, 2018). Simultaneously, this is enabled by the breakthroughs in the fields of antibody-drug conjugates or cell and gene therapy, along with the better understanding of process scale-up of recombinant protein production.

The average annual approvals of biopharmaceutical drugs in the US and EU doubled since the beginning of the century (**Figure 1.2**). Monoclonal antibodies,

recombinant hormones (e.g. insulin) and blood clotting factors (e.g. factor VIII) dominated the new biotech-based products entering the market between 2014 and 2019. Since 2020, although COVID-19 vaccines shot to the top of the list of highest-grossing individual products, monoclonal antibodies continue to lead biopharmaceuticals in numbers of approvals and sales (Walsh and Walsh, 2022).

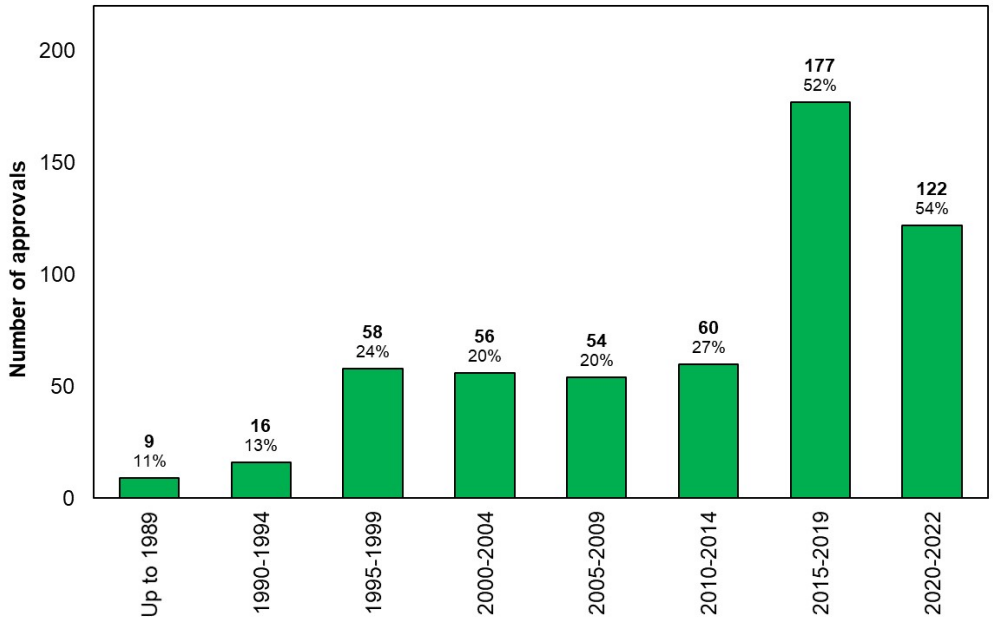

Figure 1.2 - Biopharmaceuticals approval profile up to 2022. The data labels indicate the number of total biologics approvals in that period and the percentage of mAb approvals from the total number. Sourced from (Walsh and Walsh, 2022).

Table 1.1 highlights the top 20 best-selling biologics globally in 2021, showcasing their respective product types, revenues, approval dates, and patent expiries (Walsh and Walsh, 2022).

The total sales of monoclonal antibodies in 2021 reached $217 billion, which represented more than 80% of total biopharmaceuticals sales that year. COVID vaccines ranked third on reported sales values, with $54 billion revenues (Walsh and Walsh, 2022).

As monoclonal antibodies are subjects of this thesis, further details on their characteristics and production will be presented in **Section 1.5**.

Table 1.1 - Top 20 best-selling biologics in 2021 worldwide. Sourced from (Walsh and Walsh, 2022). All products from this table are produced in a batch platform.

#	Product	Type	Revenues 2021 ($ billions)	Approval	Company	Patent Expiry
1	Comirnaty®	mRNA vaccine	36.8	2020	Pfizer & BioNTech	N/A
2	Humira®	mAb	21.2	2002	AbbVie, Eisai	2016 (US), 2018 (EU)
3	Spikevax®	mRNA vaccine	17.7	2020	Moderna	N/A
4	Keytruda®	mAb	17.2	2014	Merck	2036 (US), 2028 (EU)
5	Stelara®	mAb	9.5	2009	Janssen	2023 (US), 2024 (EU)
6	Eylea®	fusion protein	9.4	2011	Regeneron, Bayer	2027 (US), 2027 (EU)
7	Opdivo®	mAb	8.5	2014	Bristol-Myers Squibb, Ono Pharmaceutical	2027 (US), 2026 (EU)
8	Ronapreve/ Regen-Cov®	mAb	7.6	2020	Roche, Regeneron	N/A
9	Trulicity®	GLP	6.7	2014	Eli Lilly	2026 (US), 2024 (EU)
10	Darzalex®	mAb	6.0	2015	Janssen	2027 (US), 2026 (EU)
11	Dupixent®	mAb	5.9	2017	Sanofi-Aventis, Regeneron	N/A
12	Prolia/Xgeva®	mAb	5.7	2010	Amgen	2025 (US), 2022 (EU)
13	Gardasil 9®	fusion protein	5.7	2014	Merck	2028 (US), 2028 (EU)
14	Enbrel®	fusion protein	5.6	1998	Amgen, Pfizer, Takeda	2028 (US), 2015 (EU)
15	Ocrevus®	mAb	5.5	2017	Roche, Genentech	2029 (US), 2027 (EU)
16	Consentyx®	mAb	4.7	2015	Novartis	2026 (US)
17	Entyvio®	mAb	4.4	2014	Takeda	2026 (US)
18	Perjeta®	mAb	4.3	2012	Roche, Genentech	2024 (US), 2023 (EU)
19	Soliris®	mAb	4.2	2007	Alexion Pharmaceuticals	2021 (US), 2020 (EU)
20	Lantus®	insulin	3.9	2000	Sanofi	2014 (US), 2014 (EU)

1.4 Challenges in the biopharmaceutical industry

The inherent complexity of biologics and biological processes, coupled with stringent regulatory requirements within pharmaceutical companies, poses challenges across the biopharmaceutical industry. Apart from the substantial construction costs and validation demands associated with GMP facilities, expenses linked to complex analytical technologies and costly cell culture media significantly impact manufacturing costs. Synthetic drugs, synthesised through conventional chemical methods, can be produced at around $5 per gram, while biologics' production costs can range between hundreds and hundreds of thousands of dollars per gram (Sommerfeld and Strube, 2005; Farid, 2007 & 2017).

Also, the increase in product pipeline diversity associated with the growing market demand for biopharmaceutical creates the need for production flexibility, which is delicate due to the capacity adjustments associated. Likewise, scaling speed must be swift, prompting the industry to focus more on standardised production platforms (Konstantinov and Cooney, 2015). Furthermore, ensuring standardisation in biomanufacturing is particularly challenging, demanding rigorous control systems and robust manufacturing processes. Because of traditionally higher holding times of products during batch production, their quality attributes may vary, which is not desirable at a regulatory level. More recently, attention has also been given to the environmental impact of the biotech industry, with focus on the water and energy consumption and waste generated on biopharmaceuticals production (BioPhorum, 2023).

Owing to the pressing markets, the rise of biosimilars and, thus, the mission of creating more cost-effective products, biopharmaceutical companies have been working on overcoming these challenges. This includes the adoption of new production schemes, such as continuous processes, and leveraging process economic models to make informed decisions about production strategies (Yang, Qadan and Ierapetritou, 2020).

1.5 Biologics manufacture

In biologics production, the technological advances at a manufacturing level demonstrate significant potential to reduce costs, increase productivity, allow production flexibility and reduce facility footprints (Fisher et al, 2019). Workflows for the processing of biotech-based products have been established and optimised over the years. A generic process scheme for these products is presented in **Figure 1.3**.

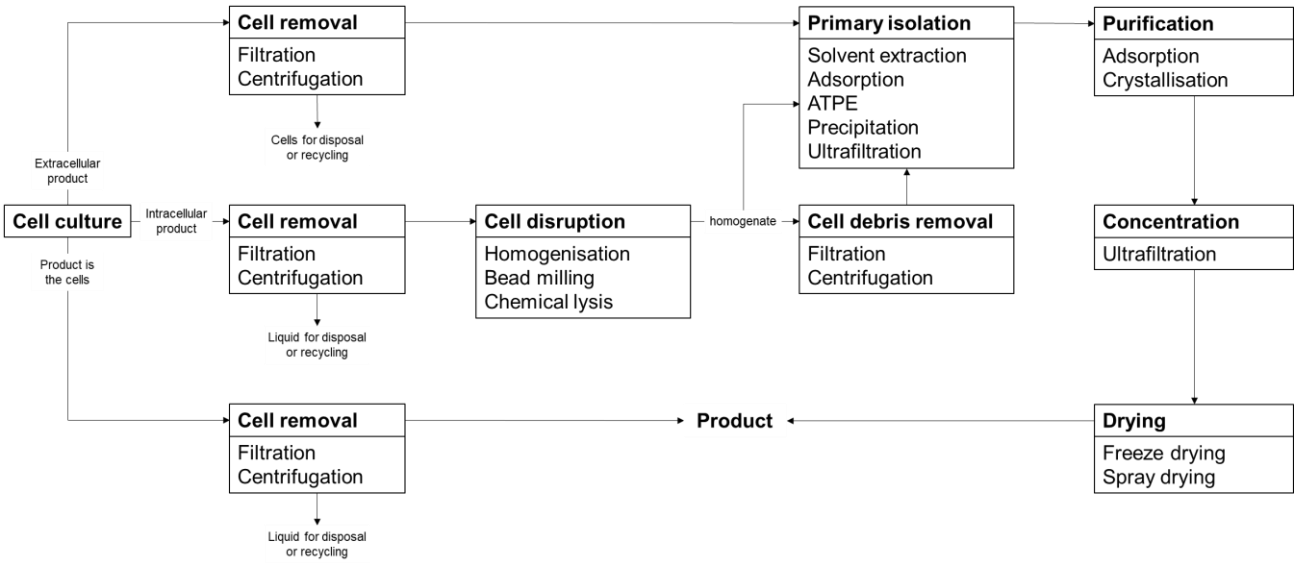

Figure 1.3 - Generic production scheme of biologics when cells are the product (e.g., probiotic products, live attenuated vaccines) or the products are located intra and extracellularly. Insulin, growth factors, monoclonal antibodies and other products can be produced both intra and extracellularly. Adapted from (Doran, 2013) with some unit operations (e.g., ATPE, precipitation) added to the original scheme.

Production characteristics and technical advances in the field of monoclonal antibodies manufacture are in the scope of this thesis and will be further reviewed in the next sections.

1.5.1 Production of monoclonal antibodies

The field of engineered mAbs derives from Köhler et al. who discovered in 1975 that single fusion cell lines (hybridomas) could be generated by fusing murine

B cells with murine myeloma cells to produce antibodies with a unique specificity, i.e. monoclonal antibodies (Köhler and Milstein, 1975). As the hybridoma technology started to lead to human anti-mouse antibodies development by the patients, a new generation of recombinant mAbs based on the production of antibodies in cellular systems arose (Kunert and Reinhart, 2016).

Reports using CHO cells for mAb expression show production yields ranging from 1 to 10 g/L using fed-batch cultures (Luan Y, 2006; Reinhart et al., 2015; Zboray et al., 2015) and titres higher than 10 g/L in perfusion modes (Liang, Luo and Li, 2023). For PER.C6, impressive results were achieved by Kuczewski et al. (2011) using a high cell density bioreactor, where 27 g/L of mAb were obtained (Kuczewski et al., 2011). CHO cells are still commonly favoured for mAb production due to their well-established track record, robustness, stability and safety (Zhang, 2014).

The most commonly used scheme for the production and purification of monoclonal antibodies is presented in **Figure 1.4**.

As mAbs are typically expressed extracellularly in mammalian systems, the first processing step after harvesting the cell culture is centrifugation followed by depth filtration to remove the biomass/large debris and clarify the resulting liquid (in batch/fed-batch based cell culture). The primary capture of the antibodies is then achieved with Protein A chromatography, which has a high selectivity for mAbs. The dynamic binding capacity of such resins vary from 15 to 100 g/L of resin and the purity achieved is typically higher than 95% (Tarrant et al., 2012; Dransart et al., 2018). In this step, impurities such as host cell proteins or DNA are removed typically in the flow through and the elution step is performed at low pH, which eliminates the need for buffer exchange or pH adjustment before the virus inactivation. Protein A resin can be re-used for several cycles (up to 200, depending on the product); nevertheless, it has some disadvantages, mainly related with the high cost of the resin, which ranges from 8000 to 15000 £/L resin (Karst *et al.*, 2017; Yamada *et al.*, 2017; Ramos-de-la-Peña, González-Valdez and Aguilar, 2019). After the virus inactivation, ion exchange chromatography takes place to remove residual impurities, including leached Protein A. Cation and anion exchange (CEX and AEX) are often in the process

in interchangeable order, depending on process requirements and constraints. Hydrophobic interaction chromatography may be also integrated to assist with aggregates removal (Shukla et al., 2017). Finally, a virus reduction filtration assures the viral safety of the product before the concentration of the product at the final UF/DF step.

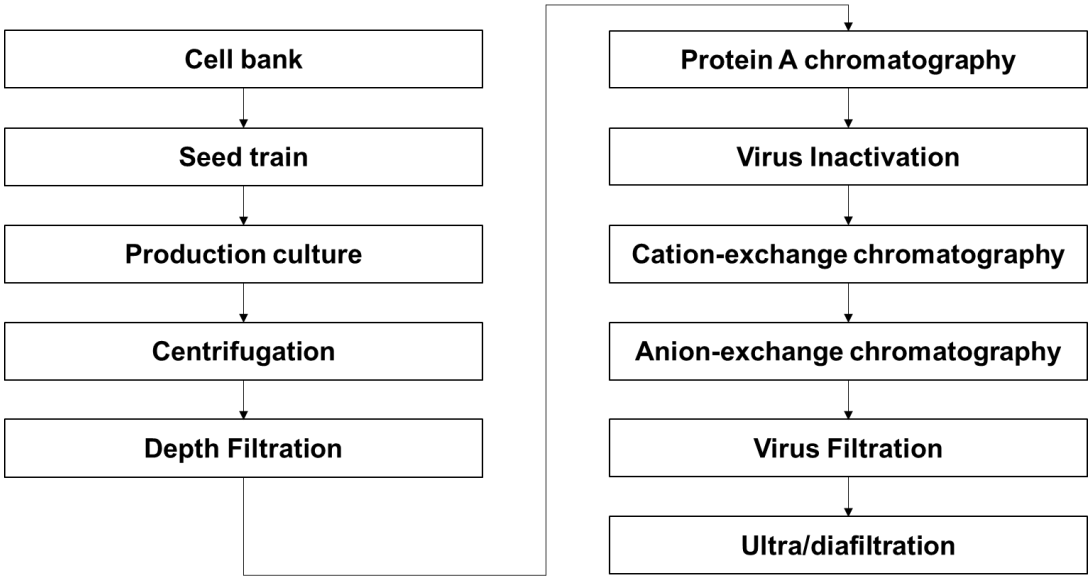

Figure 1.4 - Typical monoclonal antibodies production scheme. Cation and anion-exchange steps may be in switched order.

1.5.1.1 Continuous manufacturing of biopharmaceuticals

While continuous processing is considered a standard approach for wastewater treatment, composting and certain bioenergy processes such as bioethanol and biogas production, the production of biopharmaceuticals relies predominantly on batch processes. However, the interest in reducing the cost of biopharmaceuticals coupled with the challenges outlined in **Section 1.4** has led to a growing awareness of the advantages of continuous biomanufacturing. Examples of benefits commonly reported in the literature are: enhanced efficiency and productivity, reduced operational costs, reduced waste or more consistent product quality (Gerstweiler, Bi and Middelberg, 2021; Rathore, Thakur and Kateja, 2023). Also, regulatory authorities have been increasingly supporting this new production paradigm for biopharmaceuticals (Hernandez, 2015).

Continuous upstream technologies, such as perfusion and chemostat cell cultures, are the most common examples of continuous bioprocesses applied to biologics manufacture. Also, the emergence of continuous chromatography has paved the way for the integration of continuous upstream and downstream processing.

Examples of biopharma investment in the research and implementation of continuous technologies have been reported:

- In 2014, Amgen completed a $200 million plant in Singapore for the use of continuous purification of monoclonal antibodies (Palmer, 2014);
- In 2019, Sanofi Genzyme opened a $320 million manufacturing facility in Framingham (MA, USA) for continuous mAb production;
- In 2022, Just-Evotec inaugurated its first modular continuous manufacturing "facility of the future" in Washington, named J.POD biologics. The company is also in process of building a similar $180 million facility in Toulouse, with a projected operational date set for 2024 (Kansteiner, 2021);

Single-use (SU) technology combined with continuous processing is also a pathway that has been studied to decrease overall production costs in biopharmaceuticals production (Hummel *et al.*, 2019; Mahal, Branton and Farid, 2021). While in a batch process the increment in cost - due to the high consumables expenditure at large scales - can be discouraging (Shukla and Gottschalk, 2013), the synergy between SU and continuous technology allows for the reduction of equipment sizes (and disposables), thus, turning the application of single-use attractive. Single-use facilities may lead to cost savings related to avoidable cleaning procedures, ease of validation and consequent higher throughputs. A leading example of continuous single-use technology applied in biomanufacturing is Amgen's facility in Singapore for the production of monoclonal antibodies (Shukla *et al.*, 2017).

1.5.1.1.1 Continuous Upstream Processing

Batch commercial manufacture often requires reactors over 2000L, more commonly between 10,000 and 20,000L, which involves significant capital investments, large footprints and large energetic inputs for heating and cooling. With bioreactors operating in continuous mode, not only is the equipment required smaller (around 500 - 2000L for equivalent demands), but the operation can also be steadier and the productivity higher, as cells remain in their optimal growth and production phases for longer periods and the production downtime is minimised (Langer and Rader, 2014; Bielser *et al.*, 2018).

Efforts have been made to improve perfusion architecture and further augment the advantages of continuous technology. Improvement efforts include the increase in host cell line stability and robustness to deliver high productivities for periods of 2 to 3 months, media optimisation that can support cell densities higher than 50E6 cells/mL at perfusion rates between 1 and 2 reactor vol/day, automatic cell density control and foam control (Konstantinov and Cooney, 2015).

A list of commercialised monoclonal antibodies produced through continuous cell culture is presented in **Table 1.2**. There is limited data on the annual demand or bioreactor volumes for these products; however, personal communications with Janssen representatives indicate that these monoclonal antibodies are typically produced at lower volumes compared to those commonly seen in batch production. Interestingly, none of the examples presented in **Table 1.2** are included in the top 20 best-selling products listed in **Table 1.1**, which could reflect the cautious adoption of continuous biomanufacturing by large BioPharma companies.

Table 1.2 - Recombinant monoclonal antibodies produced via perfusion cell culture. Source: Pollock, Ho, and Farid, 2013; Chu and Robinson, 2001; Lindskog, 2018.

Product Name	Type	Approval	Company	Retention Device
ReoPro®	mAb	1994	Janssen	Spin filter
Remicade®	mAb	1998	Janssen	Spin filter
Simulect®	mAb	1998	Novartis	Rotational sieve
Simponi®	mAb	2009	Janssen	ATF
Stelara®	mAb	2009	Janssen	ATF

1.5.1.1.2 Continuous Downstream Processing

While continuous upstream is reasonably well established and thoroughly reported in literature (Henry, Kwok and Piret, 2008; Langer and Rader, 2014; Desai, 2015; Dorceus et al., 2017; Bielser et al., 2018; Kim et al., 2019), adoption of continuous downstream processes has been slow due to the ongoing maturation of the technologies and the need for proof of cost-effectiveness.

One of the main advantages of continuous DSP in bioprocesses is the decrease in hold steps and residence times (which can go up to 72h), which not only contributes to the production overall throughput, but also decreases the likelihood of degradation of unstable products (Warikoo *et al.*, 2012; Godawat *et al.*, 2015).

In conventional processes, DSP costs can range from 50 to 90% of the total cost of goods. Moreover, around two thirds of total DSP equipment costs can be attributed to chromatography units (Strube et al., 2011; Subramanian, 2012). In continuous separation processes, costs can be reduced due to the decrease in equipment size and buffer consumption (Cramer and Holstein, 2011).

Most common continuous DSP operations in mAb production include continuous chromatography steps for capture and polishing (e.g. periodic-counter current chromatography - PCC, simulated moving bed - SMB, multi-column counter-current solvent gradient continuous purification - MCSGP, continuous annular chromatography - CAC) and continuous filtration applied to virus removal, concentration and buffer exchange (e.g. single-pass tangential flow filtration - SPTFF, alternating tangential flow filtration - ATF). Continuous virus inactivation can be also achieved by having alternating tanks incubation (semi-continuous) and, most recently, Martins et al. (2019) presented a packed-bed reactor coupled with an in-line mixer which proved to be as efficient as the operation in batch (Martins et al., 2019). Also, in mAb production, Protein A elution step is commonly done at low pH, thus, continuous capture enables/facilitates the virus inactivation.

1.5.1.1.2.1 Continuous Chromatography

In chromatography, as the mass of protein to purify increases with scale, the required resin, buffer and elution volumes also escalate. Moreover, in conventional batch chromatography, the resin is not loaded to its maximum capacity to avoid losses and it is typically cycled multiple times per batch. In continuous chromatography, instead of the average 80% capacity used, columns can achieve complete saturation, as the flow-through is loaded to a second column connected (Hernandez, 2015). This process maximises productivity and reports cite improvements in resin utilisation of up to 30% (Hernandez, 2015), with associated 20 to 40% savings in resin volume and 20% in buffer (Shanley, 2017).

Periodic counter-current chromatography (PCC) was developed by GE Healthcare and uses multiple packed columns in a continuous bind-and-elute purification process. The most important focus of a PCC design is to ensure that the number of columns is so that the loading time is greater or equal to the time needed for elution, recovery and regeneration (Zydney, 2016). Simulated moving bed technology is also based on a counter-current operation, however, it is typically run with four columns assigned to four zones and provides high-

resolution separation of compounds due to molecules with different interaction strengths being eluted in different streams (Subramanian, 2018). Although PCC and SMB are the most common chromatography setups adopted in continuous processing, there are other configurations studied to provide higher productivity, smaller resin and buffer requirements and smaller footprints compared to conventional batch purification steps (Vogel et al., 2002; Aumann and Morbidelli, 2007; Muller-Spath et al., 2010). A scheme of different systems is presented in **Figure 1.5**.

As continuous chromatography is undoubtedly the most well-characterised process within continuous downstream processing, several suppliers have been making available continuous chromatography systems for the purification of biopharmaceuticals. A list of pilot and large-scale continuous chromatography equipment is shared in **Table 1.3**.

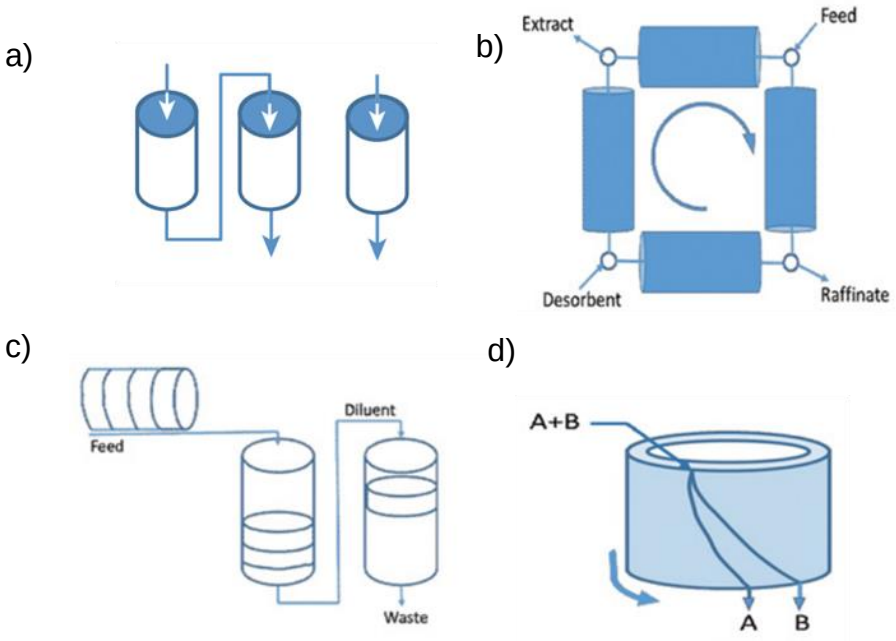

Figure 1.5 - Continuous chromatography configurations in pharmaceuticals processing. a) Periodic counter-current chromatography - PCC, b) simulated moving bed - SMB, c) multi-column solvent gradient purification – MCSGP and d) continuous annular chromatography. Source: (Jungbauer, 2013; Rathore *et al.*, 2015)

Table 1.3 - List of continuous chromatography equipment, specifications, and suppliers.

Equipment Name	Chromatography type	Columns installed (basic setup)	Flow Rates (L/h)	Supplier
AKTA PCC	PCC	4	0.03 – 2000	GE Healthcare (New Jersey, USA)
BioSC	PCC	6	0.06 – 90	Novasep (Pompey, France)
Contichrom CUBE	SMB/MCSGP	2	0.01 – 6	ChromaCon (Zurique, Switzerland)
Cadence BioSMB	SMB	8	0.06 – 350	Ex-Pall Life Sciences (New York, USA)
SembaPro	SMB	8	0.06 – 120	Semba (Wiscousin, USA)

1.5.1.1.2.2 Continuous Filtration

Single-pass tangential flow filtration (SPTFF) was designed to answer the needs of converting conventional tangential flow filtration, which involves the recirculation of liquid across a recirculation tank, into a continuous unit operation. In SPTFF, which can be used for product concentration and diafiltration, as soon as the feed reaches the filtration module, it is distributed over multiple cassettes and, in a single-pass, it must reach the desired concentration. More recently, Pall has also developed an in-line configuration of SPTFF which allows for in-line concentration (ILC) and in-line diafiltration (ILD) of the product (Dizon-Maspat et al., 2011). SPTFF can also be used in the virus filtration step, where typical instalments rely on an in-line SPTFF built with a filter for virus clearance in between the chromatography and concentration units (Clutterbuck et al., 2017).

In continuous biopharmaceuticals production, alternating tangential flow filtration (ATF) is frequently used to separate cells from the product and retain/return them in/to the perfusion. The low shear pump built in these systems prevents cells from damage, while the alternating flow maintains the flux by unblocking any clogged fibres (Zydney, 2016).

1.5.2 **Column-free purification strategies**

As mentioned in **Section 0**, despite its good performance in mAb capture, protein A affinity chromatography constitutes a very expensive step in the overall process, which has led to a longstanding interest in purification alternatives from both academia and industry. As the goal of this thesis is to investigate how competitive such alternatives can be compared with protein A chromatography, the following sections provide insights into the technical parameters and results obtained for several options.

Different choices include aqueous two-phase systems and protein precipitation, which have showed to enable higher volume feeds, thus, lowering processing times (Gronemeyer, Ditz and Strube, 2014).

1.5.2.1 Membrane Chromatography

Membrane chromatography employs membranes with immobilised ligands that facilitate selective binding of target molecules. Unlike conventional chromatographic techniques, membrane chromatography integrates the separation and purification processes into a single step, thereby streamlining production workflows. The technology is highly compatible with continuous processing, allowing for effective operation in integrated and scalable systems (Muthukumar *et al.*, 2017; Trnovec *et al.*, 2020; Chen *et al.*, 2023)

In the context of monoclonal antibody (mAb) purification, membrane chromatography has also been demonstrated (Osuofa and Husson, 2023; Schmitz, Minceva and Kampmann, 2024). For instance, Osuofa et al. (2023) have shown that membrane chromatography can achieve high purification levels with good product recovery compared to traditional methods. Specifically, membrane chromatography setups have demonstrated dynamic binding capacities of approximately 40 mg/mL, comparable to traditional resins, with faster binding times. Another example of the successful application of membrane chromatography is shared by Schmitz et al. (2024), presented a membrane chromatography system designed for mAb purification with a dynamic binding capacity up to 150 mg/mL and 95% removal of impurities.

Challenges related to membrane chromatography include membrane fouling and limited selectivity for certain feedstocks, which can impact the overall efficiency of the process. Moreover, membrane chromatography generally exhibits lower selectivity compared to Protein A affinity chromatography. This lower specificity can result in less efficient separation of antibodies from other proteins and impurities, affecting overall product purity (Ghosh, 2002).

1.5.2.2 Aqueous Two-Phase Extraction (ATPE)

The interest in aqueous two-phase extraction for biopharmaceutical processes is linked to its high biocompatibility with biomolecules (phases are 80 to 90% water), high capacity and ease of scaling-up (Rosa *et al.*, 2010; Iqbal *et al.*, 2016). Also, in contrast to other chromatographic or non-chromatography techniques, ATPE enables the purification of proteins from crude feedstocks, showing both clarification and purification/concentration functionalities. **Figure 1.6** represents a continuous ATPE scheme developed by Rosa et al. (2012).

The application of ATPE in mAb purification has been widely reported (e.g., Bras et al., 2017; Azevedo, Rosa, and Ferreira, 2008; Oelmeier, Ladd-Effio, and Hubbuch, 2013; Azevedo et al., 2007). In 2013, Rosa et al. presented a continuous ATPE setup for the purification of human IgG using a multi-stage extractor, where recovery yields between 80 and 100% and purities between 97 and 100% were obtained. The economic and environmental evaluation of ATPE in continuous mode was also performed by the researchers, who estimate 39% cost-savings comparing to conventional protein A chromatography (batch-based) (Rosa et al, 2011). More recent studies by Anupa et al. demonstrated the use of ATPE for the purification of mAbs with a PEG-sulfate sytem, achieving yields higher than 80% and purities of 97% (Anupa *et al.*, 2024). Similarly, Lohmann et al. explored a novel approach combining ATPE and precipitation for mAb capture. The integrated process resulted in a mAb recovery yield of 85% with significant impurity removal (Lohmann and Strube, 2020).

Challenges of ATPE typically relate to the purity levels reached compared with affinity chromatography and the sensitivity to feed stream variability;

nevertheless, process optimisation is ongoing and new ATPE strategies are being developed to be technical and financially competitive with current protein A capture (Low, Leary and Pujar, 2007; Gronemeyer, Ditz and Strube, 2014).

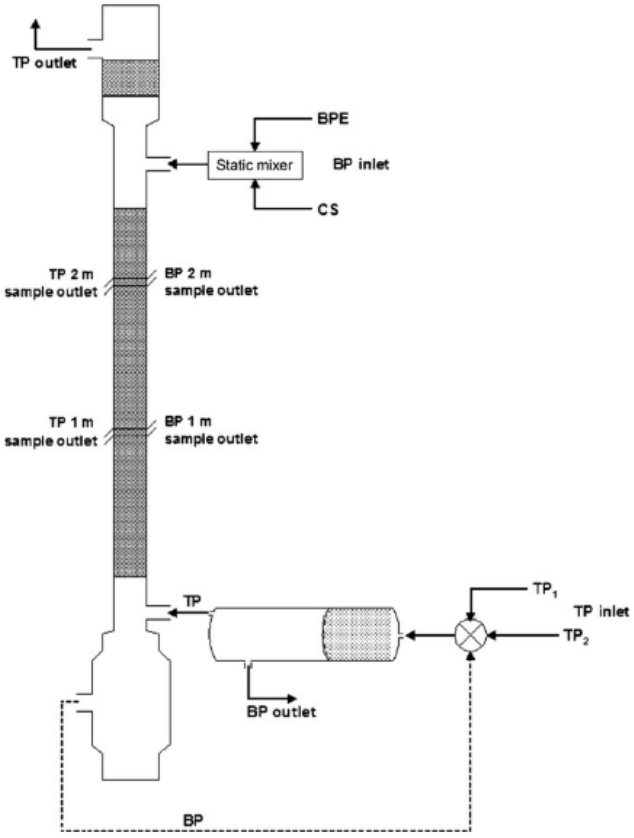

Figure 1.6 – Schematic of a glass continuous ATPE extractor (Rosa *et al.*, 2012). The column is operated at room temperature in a continuous operation mode, with the top (TP) PEG-rich phase being continuously dispersed at the bottom (BP) of the column through a capillar and the bottom phosphate-rich phase being continuously fed at the top of the column. CS stands for cells supernatant (or HCCF - harvest cell culture fluid - , as further referenced in this thesis).

1.5.2.3 Precipitation

Precipitation has been used both for purifying target products or reducing impurities in biopharmaceutical production. Common systems comprise co-precipitation of antibodies or impurities with negatively or positively charged agents, respectively (e.g., Peram et al., 2010; Ma et al., 2010; McDonald et al.,

2009). Precipitation with caprylic acid or PEG has been also broadly applied in fractionation of antibodies and blood factors from plasma (Buchacher and Curling, 2019). PEG precipitation can also be combined with isoelectric precipitation to improve separation efficiency (Lain et al., 2010).

Literature shows that precipitation is mostly suitable when feedstocks have a high titre and fairly high purity, as the selectivity of the technique is poor compared to other alternatives (Jungbauer, 2013; Li et al., 2019); nevertheless, precipitation in continuous mode is an interesting option in non-protein A platforms for mAb production. In 2014, Hammerschmidt et al. modelled a series of precipitation steps in a fully continuous recombinant mAb production scheme without protein A affinity chromatography and performed an economic analysis that showed that the operation was cost-competitive with a conventional mAb batch process at titres higher than 6 g/L (**Figure 1.7a**). Kateja et al. presented continuous precipitation in a coiled flow inverter reactor (**Figure 1.7b**) and obtained 7 times higher productivities and 5 times lower cycle times and equipment utilisation comparing to batch processes, with a recovery yield higher than 95% for the step (Kateja et al., 2016 & 2018). Following the work of Burgstaller and Satzer (2019), Li et al. (2019) used zinc chloride coupled with PEG to precipitate mAbs in a tubular reactor directly from harvested cell culture, showing a fully integrated continuous process for precipitation, dewatering, washing, and mAb resolubilisation with an overall product recovery of 80% (**Figure 1.7c**).

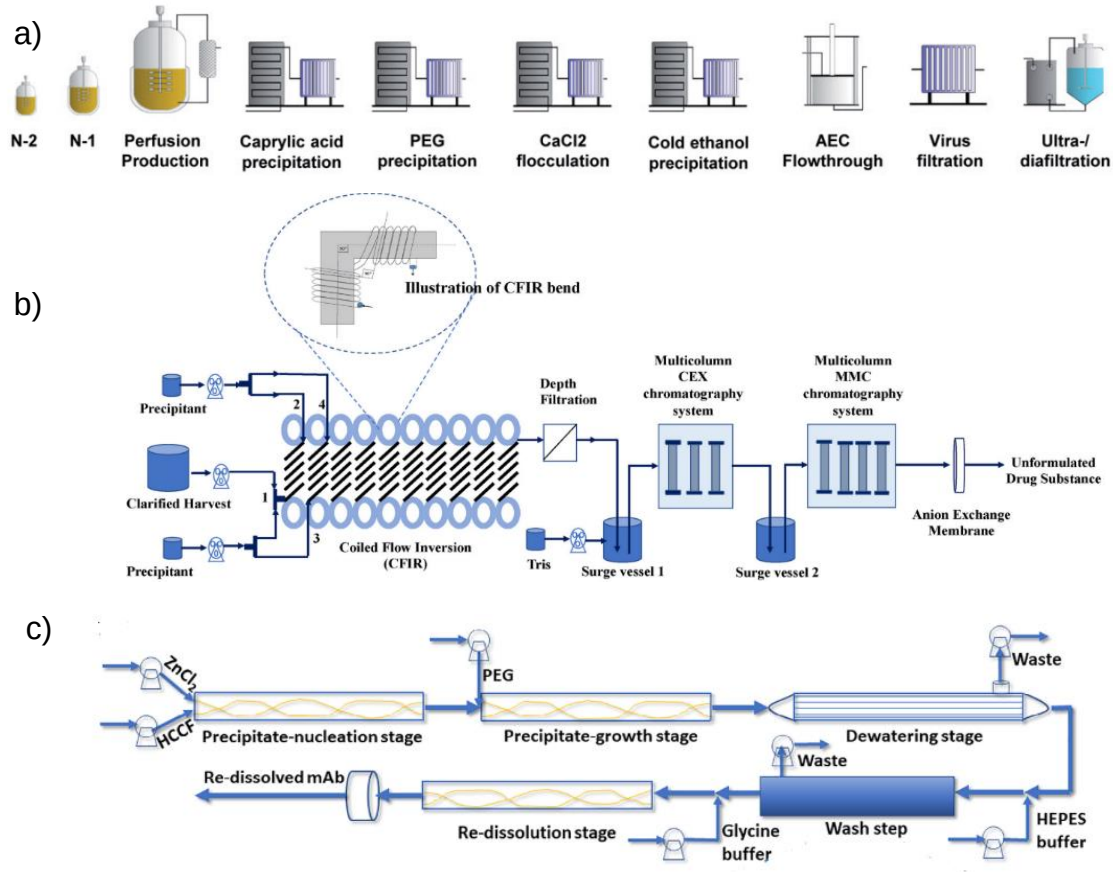

Figure 1.7 - Fully continuous non-Protein A flowsheets for antibodies production with integrated purification by precipitation. a) Series of four precipitation steps presented by (Hammerschmidt et al., 2014), where caprylic and $CaCl_2$ precipitate contaminants and PEG and ethanol precipitate the product. b) Coiled flow inversion reactor presented by (Kateja et al., 2016) with the same precipitation steps than previous scheme, but with several pumping strategies to deliver the reagents inside the coiled reactor. c) Continuous precipitation system coupled with counter current tangential flow filtration for mAb separation, dewatering and washing (Li et al., 2019).

1.5.2.4 Crystallisation

Crystallisation is a technique that can be for purifying and concentrating monoclonal antibodies and involves inducing the formation of a solid crystalline phase from a protein solution. This method leverages the unique physicochemical properties of molecules to achieve high purity and

concentration, making it particularly suitable for final product polishing and formulation (Chougale *et al.*, 2023).

While both precipitation and crystallisation techniques aim to separate the target molecule from a solution by changing its conformation, crystallisation typically results in higher purity and concentration due to the selective nature of crystal formation (Pu and Hadinoto, 2020). In contrast, precipitation often involves less selective aggregation of proteins and impurities, which might require additional purification steps, but generally results in higher yields.

Several studies have demonstrated the application of crystallisation in mAb production. For example, Rajoub et al. demonstrated that conventional protein A chromatography could be replaced with a single crystallisation step. The process achieved a final purity of 98% and a recovery yield of 85% (Rajoub *et al.*, 2023).

Nevertheless, the main challenge regarding crystallisation lies on the optimisation of conditions for effective crystallisation, which can be complex and time-consuming, as it requires careful adjustment of numerous factors (e.g., temperature, pH, concentration) to achieve high-quality crystals (Chen *et al.*, 2021).

1.5.3 Environmental analysis of biomanufacturing

Recent attention has also been directed toward the environmental impact of the biopharmaceutical industry, with emphasis on water and energy consumption as well as waste generation during production.

This emphasis on sustainability has driven the integration of environmental analysis into biomanufacturing processes. Two key metrics, Process Mass Intensity (PMI) and Life Cycle Assessment (LCA) outputs, have been playing a pivotal role in assessing and optimising the environmental performance of biomanufacturing processes.

1.5.3.1 Process Mass Intensity (PMI)

PMI is a straightforward metric that gauges the utilisation of raw materials during a manufacturing process relative to the resulting product output. This metric offers valuable insights into resource efficiency and waste generation of a production scheme. Madabhushi et al. (2018) and Cataldo et al. (2020) have also used the PMI to identify the main sources of waste generation and most environmentally friendly process alternatives. Another metric, E-factor, is also often used when evaluating the environmental impact of biochemical processes. However, while the PMI relates to the total mass of all materials used in the process, including the part corresponding to the product stream, the E-factor is defined as the ratio of mass of waste generated to the mass of the desired produc,t and excludes the components of the product stream.

Typical PMIs for batch and continuous antibody manufacturing flowsheets have been reported and present a wide range of values. **Table 1.4** presents the PMI values found across the literature.

However, due to their simplicity, both PMI and E-factor do have their limitations, namely the inability to provide insights on the environmental impact coming from the facility energy usage or the footprint associated to single-use plastics consumption. Also, with novel policies regarding companies' sustainability, especially on carbon emissions, they lack the broader analysis needed from the biopharmaceutical sector to answer to these concerns.

Table 1.4 - PMI values found in literature for batch and continuous mAb flowsheets. SS: stainless steel; SU: single use.

Reference	Scale (kg/year)	Assumptions	Batch PMIs		Continuous PMIs	
			Water (kg/kg)	Consum. (kg/kg)	Water kg/kg)	Consum. (kg/kg)
Ho et al. (2011)	N/A	• Batch SS • Cleaning water of tanks not included	3000 – 5000	-	4000 – 6000	-
Bunnak et al. (2016)	28	• Batch & Conti SS • Cleaning water of all tanks included	40000	25	54000	46
Pollock et al. (2017)	100 – 1000	• Batch SS • Conti SU • Cleaning water of preparation tanks not included	3900 – 7250	6 – 73	2300 – 5550	8-25
Madabhushi et al. (2018)	N/A	• Batch SS • Conti SS with AEX SU • Cleaning water of tanks not included	2600	140	1900	100
Cataldo et al. (2020)	266 – 1000	• Batch SS • Conti SU for smaller scales and SS for larger scales • No information about cleaning of tanks	7000	N/A	3000 – 7000	N/A

1.5.3.2 Life Cycle Assessment (LCA)

LCA is a more comprehensive methodology that analyses the environmental impact of a product or process across its entire life cycle, encompassing stages from raw material extraction to end-of-life disposal. It can be used to assess not only water or consumables usage, but other factors, such as resource depletion, energy consumption and greenhouse gas emissions.

Figure 1.8 shows the main stages followed in a typical LCA study.

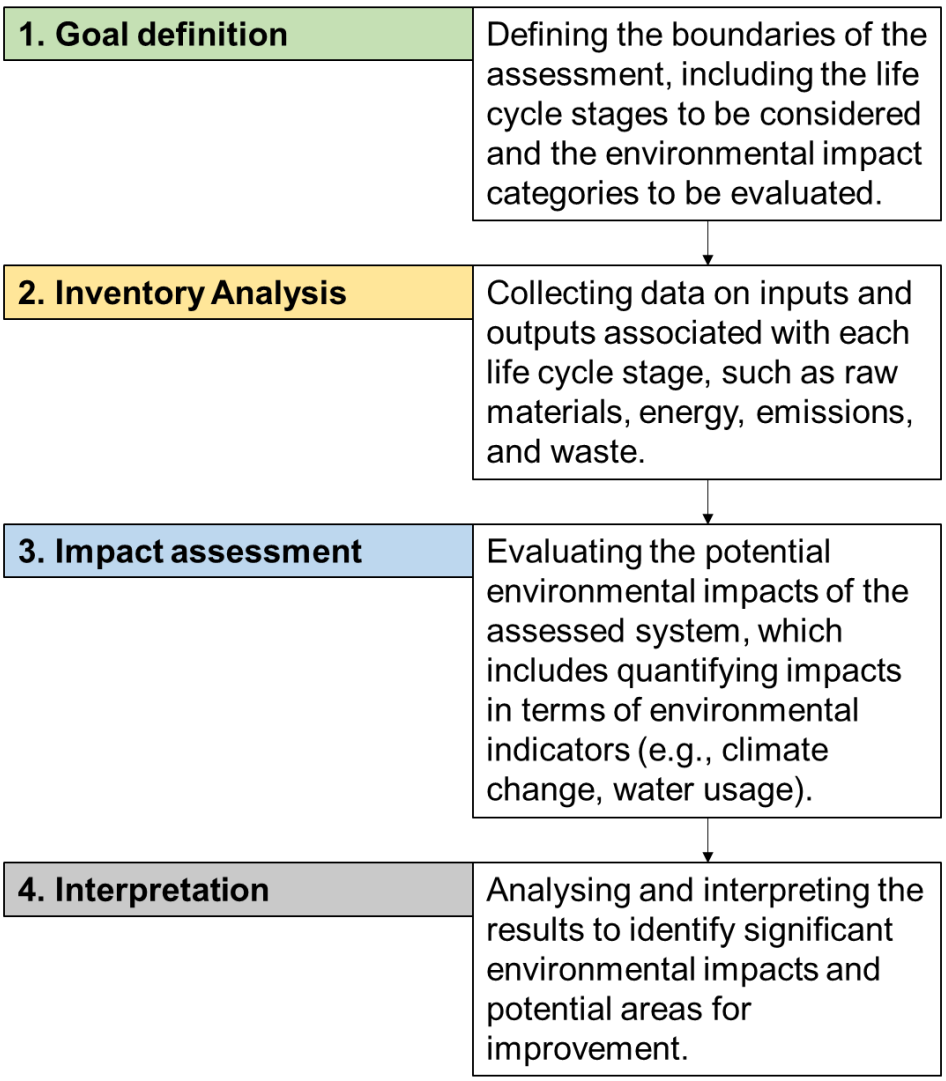

Figure 1.8 - LCA workflow from goal definition to interpretation of results.

1.5.3.2.1 Goal and scope in LCA

In the context of the life cycle assessment, defining the goal refers to establishing the specific objectives and purposes of conducting the assessment for a particular process. By clearly defining the goal upfront, one can ensure that the assessment is tailored to meet specific objectives and address the needs of the stakeholders (BioPhorum, 2023). For instance, different LCA goals may involve optimising the environmental performance of a process, or comparing the environmental outputs of different alternatives, or simply analysing the environmental markers of a specific process.

The scope of an LCA defines the boundaries and parameters of the assessment, including what processes and inputs are included and excluded. This encompasses defining the functional unit (e.g., number of batches, kg of mAb), in which the performance of the process is based, and establishing the system boundaries, which delineate the stages of the life cycle to be considered. The system boundary is a fundamental concept, as its choice significantly impacts the accuracy of the LCA results. The most typical system boundaries are described in **Figure 1.9**.

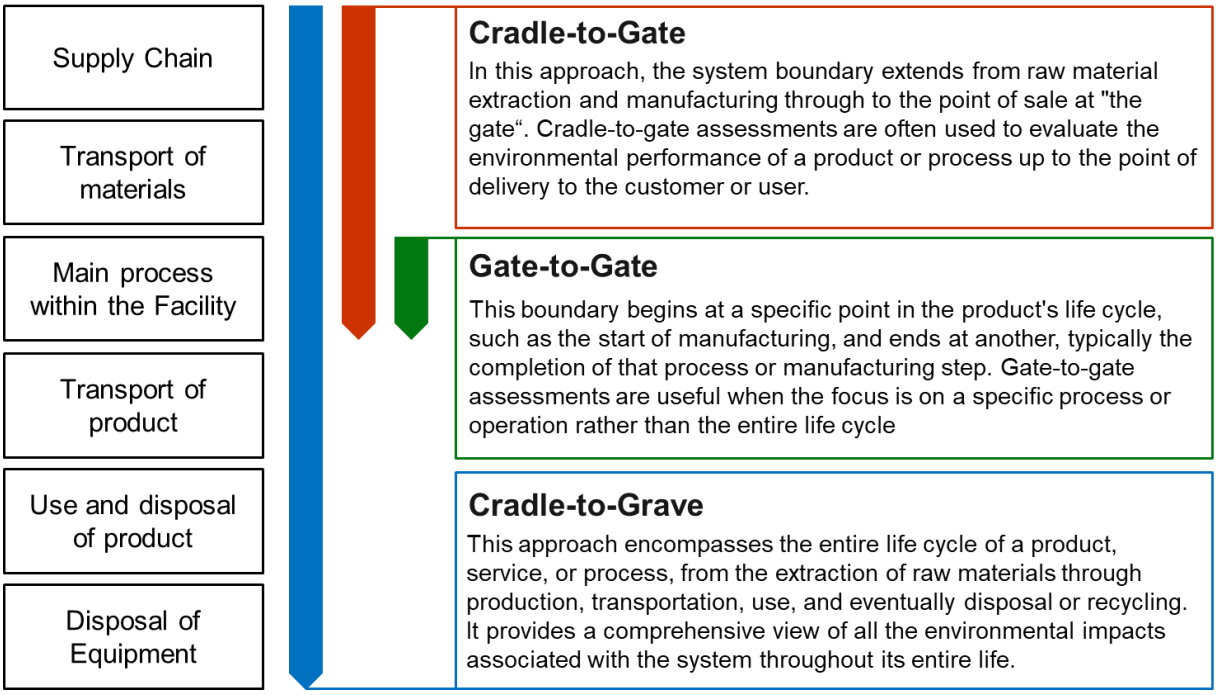

Figure 1.9 - System boundaries in an LCA study.

Additionally, the scope outlines the allocation procedures, data sources, and assumptions used in the assessment to ensure consistency and accuracy. The impact categories of interest are also identified based on the goals. A list covering the most common impact categories is showed below (ISO, 2006a & 2006b):

- Global Warming Potential (GWP): Measures the contribution to climate change in terms of equivalent carbon dioxide emissions.
- Ozone Depletion Potential (ODP): Assesses the potential for chemicals to deplete the ozone layer in the atmosphere.

- Acidification Potential (AP): Evaluates the contribution to acid rain and acidification of ecosystems.
- Eutrophication Potential (EP): Measures the potential for nutrient enrichment leading to excessive growth of algae and aquatic plant species, which can harm ecosystems.
- Human Toxicity Potential (HTP): Assesses the potential for exposure to toxic substances and their impacts on human health.
- Freshwater Ecotoxicity Potential (FEP): Measures the potential for chemicals to harm freshwater ecosystems and aquatic life.
- Terrestrial Ecotoxicity Potential (TEP): Evaluates the potential for chemicals to harm terrestrial ecosystems and organisms.
- Marine Ecotoxicity Potential (MEP): Assesses the potential for chemicals to harm marine ecosystems and organisms.
- Photochemical Ozone Formation Potential (POFP): Measures the potential for chemicals to contribute to the formation of ground-level ozone and smog.
- Depletion of Abiotic Resources: Evaluates the depletion of non-renewable resources, such as minerals and fossil fuels.
- Depletion of Fossil Energy Resources: Assesses the consumption of fossil fuels and their contribution to resource depletion.
- Water Consumption: Measures the amount of water withdrawn or consumed during a life cycle.
- Land Use: Evaluates the amount of land area required for a particular activity, including agriculture, forestry, and infrastructure.
- Occupational Health and Safety: Assesses the risks to workers' health and safety associated with a product or process.
- Noise Pollution: Measures the contribution to noise pollution from various activities or processes.

- Visual Impact: Assesses the aesthetic impact of a product or process on landscapes and views.

In this thesis, only the climate change impact category was assessed, as it represents a critical and widely recognised environmental concern. The decision not to disclose the values for other impact categories was based on the reliability and robustness of the underlying data. The carbon emission inputs were thoroughly reviewed in collaboration with project partners to ensure their relevance. However, the available data for other impact categories did not meet the same level of scrutiny, raising concerns about their consistency and potential to produce misleading conclusions.

1.5.3.2.2 Scopes of greenhouse gas emissions

The Greenhouse Gas Protocol, developed by the World Resources Institute and the World Business Council for Sustainable Development, outlines three scopes of emissions to categorise greenhouse gas emissions associated with the activities within an organisation. By categorising emissions into these scopes, companies can better understand the full extent of their greenhouse gas footprint and identify opportunities for emissions reduction and mitigation throughout their operations and value chain (WBCSD and WRI, 2012).

Each scope encompasses different stages and types of environmental impacts.

- Scope 1 - Direct Emissions

Scope 1 focuses on direct emissions from sources that are owned or controlled by the biopharmaceutical company itself. In the biopharma context, Scope 1 emissions might include energy consumption associated with the operation of facilities, including manufacturing, heating, cooling, lighting and waste generated on-site, including hazardous waste and non-hazardous waste.

- Scope 2 - Indirect Emissions

Scope 2 focuses on indirect emissions that result from the generation of purchased electricity, heat, or steam used by the biopharmaceutical company. These emissions occur outside the company's control but are associated with its energy consumption.

- Scope 3 - Indirect Value Chain Emissions

Scope 3 involves a broader perspective, looking at indirect emissions occurring outside of the company's operations. These emissions are often more challenging to quantify and manage because they involve various suppliers, customers, and other stakeholders. In the context of biopharma, Scope 3 emissions might include emissions during raw materials production, distribution and emissions from the disposal, recycling, or treatment of products at the end of their life cycle. Scope 3 emissions often have a significant impact on a product's overall environmental footprint but can be complex to assess due to the multitude of external factors involved.

1.5.3.2.3 LCA in the Biopharmaceutical Sector

The health sector contributes with around 4-5% for the total global emissions, primarily due to supply chain operations and the global shipping of equipment and medicines (Nelson, 2023). Thus, large pharmaceutical companies like GSK, AstraZeneca, and Roche, and academia are launching sustainability programmes, indicating their serious commitment to addressing sustainability challenges (Nelson, 2023). Also, CO2 emissions taxes currently in-place are providing a financial incentive for businesses to reduce their carbon emissions.

From the several impact categories provided by LCA (ISO, 2006a, 2006b), the Global Warming Potential (GWP) has been the one more commonly assessed and shared by industry to convey its sustainability efforts. GWP measures the potential for global warming caused by greenhouse gas emissions, reported in CO2-equivalents, and helps in understanding the relative environmental burden of various greenhouse gases. Also, carbon footprint, and more specifically Product Carbon Footprint (PCF), as the quantitative measure of actual emissions linked to specific activity, is typically used when referring to the environmental impact of a product. Armed with this information, industry can implement strategies to minimise their footprint and contribute to overall environmental sustainability, while complying with governmental regulation and protecting their reputation (BioPhorum, 2023).

LCA has been already used and reported in different biomanufacturing contexts (**Table 1.5**). The impact of single-use systems versus conventional stainless-steel facilities has been evaluated and it has been shown that SU flowsheets can contribute to reducing the environmental impact of biopharmaceutical products (e.g., Sinclair et al., 2008; Pietrzykowski et al., 2013; Budzinski et al., 2022). Also, Bunnak et al. (2016) conducted an LCA and cost assessment of fed-batch and perfusion (stainless-steel based) manufacturing processes for monoclonal antibodies, highlighting the trade-offs between environmental impact and manufacturing costs associated with different process strategies. The studies presented in **Table 1.5** used the raw materials (e.g., media, buffers), energy consumption, water consumption and waste generation taken from modelling different flowsheets as input to their lifecycle assessment.

Table 1.5 - System boundary of the different LCA studies found in literature in the biopharmaceutical space.

Case study (Reference)	**System boundary**
Batch SS & SU (Sinclair et al., 2008)	Cradle-to-grave
Batch SS & SU (Pietrzykowski et al., 2013)	Cradle-to-grave
FB SS & perfusion SS (batch DSP) (Bunnak et al., 2016)	Cradle-to-gate
Perfusion SS & perfusion SU (batch DSP) (Renteria Gamis et al., 2019)	Cradle-to-gate
FB SS, FB SU, perfusion SS & perfusion SU (Amasawa et al., 2021)	Gate-to-gate
Batch SU (Budzinski et al., 2022)	Cradle-to-gate

Nevertheless, the biopharmaceutical sector is lacking reports on a full and comprehensive environmental assessment of different batch and end-to-end

continuous manufacturing processes. A Product Carbon Footprint evaluation from raw material production to waste disposal, encompassing all three analysis scopes (Scopes 1, 2 and 3) and integrating industry-based assumptions for facility requirements (e.g., energy) and state-of-the-art flowsheets (e.g., end-to-end SU continuous strategies) would provide important insights for a more informed and environmentally friendly decision-making.

1.5.4 Process Analytical Technologies in biomanufacturing

Process Analytical Technologies (PAT) have emerged as vital tools in biomanufacturing, aiming to provide real-time insights and control over critical parameters and allowing for the promptly adjustment of process conditions. As these technologies have a strong foundation in the Quality by Design (QbD) initiative introduced by the U.S. Food and Drug Administration (FDA) in 2004, they are used to improve pharmaceutical manufacture by integrating analytical tools, sensor technologies, and data analysis methods into the manufacturing process (Rathore et al., 2008; Gillespie et al., 2022; Rathore, Jesubalan and Thakur, 2022). Also, with the emergence of Industry 4.0 that emphasises the integration of digital technologies, data-driven processes and smart manufacture, PAT contributes with advanced analytics and interconnectedness of machines, systems, and processes (Chen et al., 2020; Narayanan et al., 2020; Wasalathanthri, Rehmann, et al., 2020).

As the biopharmaceutical industry undergoes technology advances with the integration of end-to-end continuous manufacture strategies, it must also ensure product quality, safety and production efficiency targets are met. PAT's ability to provide real-time data supports regulatory compliance and aligns with integrated continuous bioprocessing (ICB) demands on control and consistency, while enabling possible cost savings. **Table 1.6** summarises the main economic benefits that can be provided by integrating PAT in integrated continuous bioprocessing.

Table 1.6 - Key improvements from implementing process analytical technologies in biomanufacturing.

Benefit	Impact to ICB	References
Reduce batch failure	PAT provides immediate insights into the process behaviour, enabling rapid adjustments to process conditions and minimising the risk of producing off-specification products. Rapid aggregation, glycosylation and glucose measurements have proved to be crucial to anticipate out-of-spec batches.	Patel *et al.*, 2017; Czeterko *et al.*, 2018; Feidl *et al.*, 2019; M. Y. Li *et al.*, 2019; Goldrick *et al.*, 2020; Rafferty *et al.*, 2020; Schwarz *et al.*, 2022
Improve process performance	The integration of PAT further enhances process performance by providing a continuous stream of data for monitoring and control. Augmented cell culture productivities, increases in resin utilisation or decrease in buffer consumption are some examples of PAT economic benefits on the process performance front.	Ozturk et al., 1997 Virtanen et al., 2017 Brunner et al., 2019 Goldrick et al., 2019 Moore, Sanford and Zhang, 2019 Löfgren et al., 2021 Esmonde-White, Cuellar and Lewis, 2022 Tiwari et al., 2023
Reduce QCQA and labour costs	The real-time monitoring and control offered by PAT contribute to further reducing in-process (and release) testing and, thus, materials and labour costs.	Bro, Kwiatkowski and Tolstrup, 2018 Wasalathanthri, Feroz, et al., 2020 Gomis-Fons et al., 2020 Schmidt et al., 2021 Schwarz et al., 2022

Apart from reducing batch failure, improving process performance or reducing QCQA costs at commercial scale, PAT can also enhance the understanding of critical parameters and their impact on product quality attributes in ICB. Examples of PAT utilisation in process development to gain process understanding and build robust manufacturing strategies have also been

shared in literature (e.g., Metze, 2020; Wasalathanthri et al., 2020; Santos et al., 2019; Sokolov et al., 2021).

Several PAT techniques have been integrated in continuous biomanufacturing. These techniques include spectroscopic techniques, chromatography and other sensors with multivariate data analysis (MVDA).

Spectroscopic techniques, including Infrared (IR), Raman, and Near-Infrared (NIR) spectroscopy, have gained prominence due to their non-invasive and real-time monitoring capabilities. In continuous cell culture processes, IR and Raman spectroscopy are used for monitoring cellular metabolic activity, nutrient consumption, and the production of metabolites (Rathore et al., 2015; Goldrick et al., 2020). NIR spectroscopy is employed for rapid analysis of critical parameters such as nutrient concentration, cell density, and product quality attributes during fermentation (Vogelsang et al., 2019).

Chromatographic techniques, such as High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS), are essentially used for analysing product purity and post-translational modifications in continuous biomanufacturing processes (Patel et al., 2017; Chemmalil et al., 2021).

Mass spectrometry (MS) techniques are known for their ability to provide comprehensive information on protein composition, modifications, and impurities. Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight (MALDI-TOF) and LC-MS are extensively used for peptide mapping, protein identification, and characterisation of post-translational modifications (Goh et al., 2020; Bose et al., 2022).

On the sensors front, biomass and viability probes are commonly used in real-time monitoring in continuous biomanufacturing. Capacitance, dielectric, and impedance-based sensors enable the assessment of cell viability, growth kinetics, and biomass concentration, facilitating timely adjustments to culture conditions (Konakovsky et al., 2015; Metze et al., 2020).

In continuous biomanufacturing, the integration of PAT techniques is also accompanied by advanced multivariate data analysis methods. MVDA is employed to extract meaningful information, interpret complex data, detect

patterns, and enable real-time process monitoring, fault detection, and deviation prediction (Mercier et al., 2013; Goldrick et al., 2017; Wasalathanthri, Rehmann, et al., 2020). Also, with the advances in sensor technologies and MVDA models, the inception of digital twins in continuous manufacturing of biopharmaceuticals marked a transformative phase in the industry, introducing innovative approaches to enhance process efficiency and adaptability (Park et al., 2021; Rathore et al., 2021; Gerzon, Sheng and Kirkitadze, 2022; Schmidt et al., 2022). Digital twins are data-driven, virtual and dynamic representations of bioprocesses and mirror their real action, enabling real-time monitoring, analysis, and optimisation. Ultimately, these digital replicas aim to enable model-based process control, and recent studies have showed their integration in small-scale end-to-end continuous manufacturing of mAbs with remarkable impacts on attaining consistent product quality (e.g., low HCP levels and low aggregate levels) with reduced labour (Gomis-Fons et al., 2020; Tiwari et al., 2023).

1.6 Decision-support tools in biomanufacturing

The inception of decision-support tools as a mesh of human judgement and powerful computer algorithms dates from the mid-70's (Keen, 1987). Since then, these tools have been used across agriculture (e.g., Kure, Thysen and Kristense, 1997; Rose et al., 2016), chemical (e.g., Grunow and Gunther, 2008), food (e.g., Arason et al., 2010), health care (e.g., Bryan and Boren, 2008) and construction (e.g., Shen and Chung, 2002) industries, supporting the design, optimisation, evaluation and planning of procedures.

Over the last 40 years, computer-aided simulation tools have been developed to capture both business and technical features of biomanufacturing, mainly focusing on biopharmaceutical production, as the fastest growing biotech-based market. Insights into the impact of process modifications on the capacity, cost of goods and environmental footprint of existing manufacturing facilities have been demonstrated (e.g., George, Titchener-Hooker and Farid, 2007; Liu et al., 2013; Bunnak et al., 2016; Pollock et al., 2017; Mahal, Branton and Farid, 2021) and the awareness for sources of uncertainties (e.g., batch titre, step

yields) in biomanufacturing has already been raised and integrated in these tools (e.g., Farid, Washbrook and Titchener-Hooker, 2005; Rajapakse, Titchener-Hooker and Farid, 2005; Pollock, Ho and Farid, 2013; Lyle et al., 2023; Neves, Coffman and Farid, 2024).

This chapter will cover software and methodologies used in the design of decision-support tools in biomanufacturing, as well as relevant industrial case studies to which these tools were applied. As the goal of the present research is to assess continuous bioprocesses from economic, environmental and robustness perspectives, this review aims to highlight the benefits from integrating technical and business computer simulation to expedite the evaluation of alternatives.

1.6.1 Bioprocess software tools and mathematical programming used in process economic models

As the design and performance of key unit operations can be described mathematically, the characterisation of bioprocesses can typically be done *in silico*. The choice of the most suitable software or programming languages to build bioprocess models is done according to the most important characteristics required by the user, such as flexibility, easy-to-use interface, in-built databases or the capability of performing uncertainty analysis. In the need of modelling novel production schemes, assessing the robustness of different alternatives and implementing user-defined equations and methodologies, tools which provide a higher level of flexibility will be prioritised. This section reviews several off-the-shelf process economics tools and mathematical languages and discusses the main differences, advantages and drawbacks of each option.

Different software tools for process design, economic analysis and scheduling commercially available include SuperPro Designer (SPD) (Intelligen, Scotch Plains, NJ, USA), aspenONE (Aspen Technology Inc., Cambridge, MA, USA) and BioSolve Process (Biopharm Services Ltd, Birmingham, UK). SPD and aspenONE are flowsheet-driven simulators that facilitate mass and energy balances, equipment sizing, cost analysis, debottlenecking, scheduling and

environmental impact assessment of modelled processes (Rouf et al., 2001; Petrides and Siletti, 2005). One of the main advantages of these tools is to integrate built-in process economic models and databases for raw materials, consumables and equipment costs. However, these software lack flexibility when considering the creation of user-defined models or the use of probability distributions to represent the uncertainty in process parameters (Mustafa et al., 2004). The coupling of SuperPro and Matlab was reported to enable uncertainty analysis and optimisation of bioprocesses (Taraş and Alexandria Woinaroschy, 2011; Brunet et al., 2012), yet, restrictions related with the availability of variables to be selected were encountered revealing the lack of flexibility of this methodology. BioSolve is an Excel-based process and cost modelling software and shows a higher level of flexibility compared to SPD and aspenONE, since it can perform multiple process comparisons (e.g., Torres-Acosta et al., 2016; Sinclair and Monge, 2002 & 2010; Whitford, 2018). The application of BioSolve has been regular in the most recent years when evaluating and comparing continuous bioprocessing with batch (e.g., Pollard et al. 2016; Walther et al. 2015; Hummel et al. 2019). However, this software is unable to capture dynamic modelling, such as the impact of delays, and lacks built-in features for uncertainty analysis (Torres-Acosta et al., 2015 & 2016).

Process economic models developed in programming languages as C-Sharp/C# (e.g., Simaria et al., 2014; Hassan et al., 2015) and Python (e.g., Cortes-Pena, 2019; Mahal, Branton and Farid, 2021; Lyle et al., 2023) have been reported and illustrate the versatility and extended capabilities of these tools with regard to process modelling, uncertainty analysis and application of optimisation algorithms. Although targeted bioprocess simulation tools, such as SPD, aspenONE or BioSolve can be beneficial due to the support given in integrating specific and pre-characterised unit operations in a process queue, the usage of generic mathematical packages can offer the additional flexibility and statistical power needed when simulating modifications and analysing their impact in a given process. Also, in *fit-to-purpose* models, where all operations, resources, allocation and methods are user-defined, the biomanufacturing facility can be customised and designed based on project priorities (e.g., sizing or rating mode).

1.6.2 Scope of Decision-Support Tools

The research into decision-support tools for bioprocesses generally focuses on supporting the decision-making on i) process synthesis and facility design and/or ii) portfolio management and capacity planning levels (Farid, 2012). In the first realm, the performance of different facility and process designs involves analysing economic metrics, throughput, and risk. Typically, various process sequences or unit operations are modelled in prototypes of single or multi-product facilities, and a range of sizing strategies can be chosen based on the decision-makers' priorities or preferences. As for portfolio management and capacity planning, the scope includes optimising the planning of development, manufacturing, and commercial activities for various modalities, as well as making decisions about building versus buying capacity for in-house and contract manufacturing facilities (Farid, 2012).

The different methodologies and stages used in the support to decision-making at both process design and portfolio/capacity management levels are explored in the subsequent sections and inspire the workflow followed throughout the research project.

1.6.2.1 Process Economics

The foundation of decision-support tools is process economics models. These models are designed to execute detailed mass balances and equipment sizing for every unit within the bioprocess and compute the economic (and environmental) indicators for the overall process. **Figure 1.10** summarises the framework of these models.

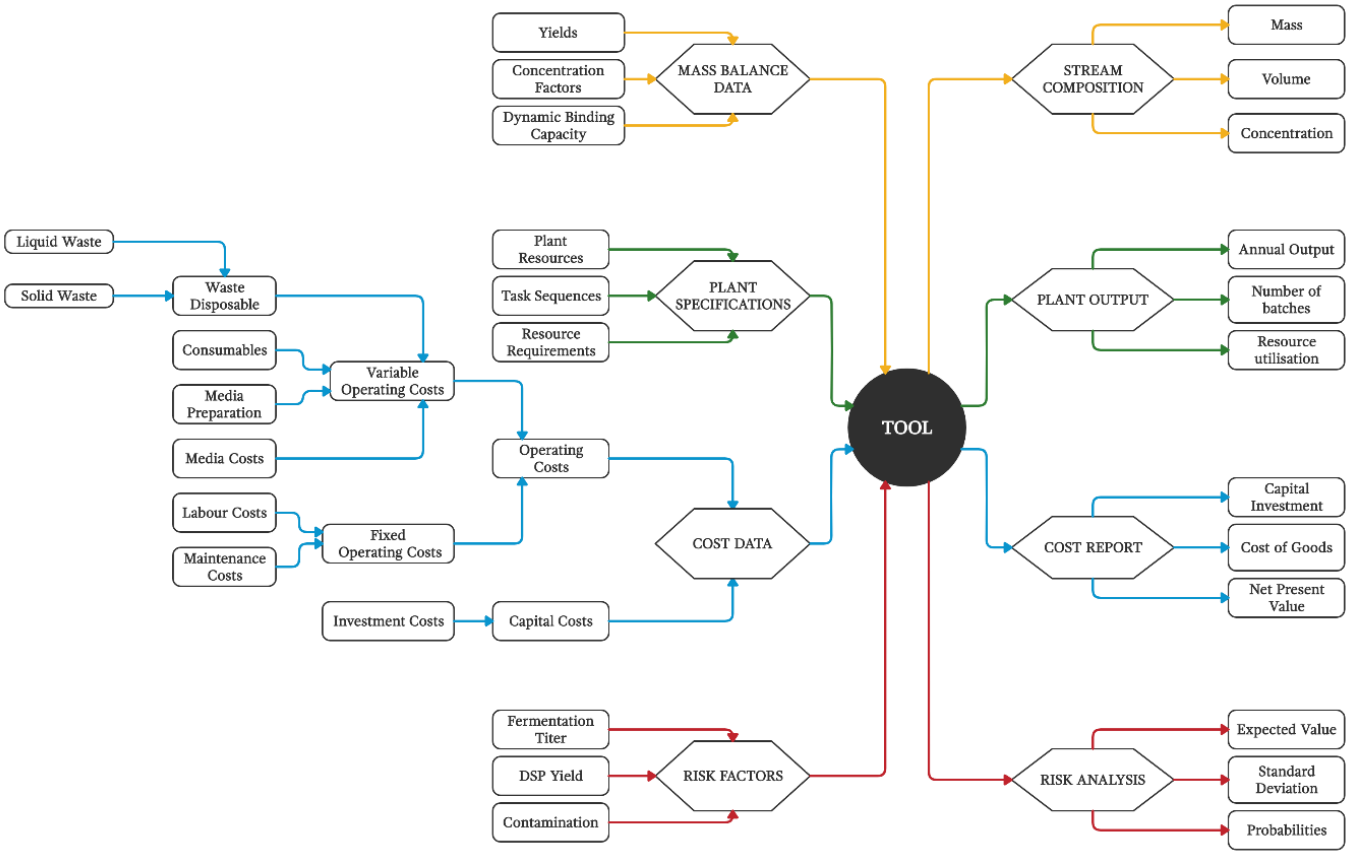

Figure 1.10 - Process economics model decomposition with key input and output parameters. Adapted from Lim et al. (2005).

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While the first step of a decision-making process deals with computing the process economics based on deterministic modelling (i.e., single defined conditions), the understanding of robustness includes the application of further methods. The confidence during decision-making is supported by accounting for uncertainties in the process (Monte Carlo simulation) or reconciling different criteria and priorities (multi-criteria decision making). The analysis workflow is shown in **Figure 1.11** and some of the tools are described in the following chapters.

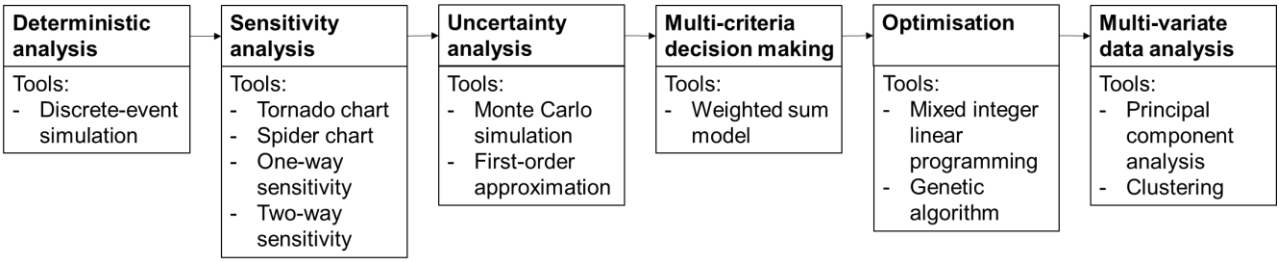


Figure 1.11 - Stages of alternatives analysis with the application of decision-support tools.

1.6.2.1.1 Monte Carlo Simulation

In every large-scale bioprocess manufacturing there are inherent uncertainties, thus, it is important to identify the key sources for technical deviations and account for them in the cost model to generate representative results. The application of stochastic modelling with Monte Carlo simulation allows to capture the effect of these uncertainties. Random values following Gaussian, triangular or Poisson distributions can be generated for certain parameters (e.g.: titres after fermentation, yields, duration of the manufacturing tasks, annual demands) and the impact on the decision-making is assessed by the likelihood of outputs exceeding certain threshold values (e.g.: cost of goods per gram of product) (Farid, Washbrook and Titchener-Hooker, 2005b).

At the process design level, Monte Carlo simulation has been used to capture the robustness of different upstream (e.g., Lim et al., 2005; Pollock, Ho and Farid, 2013) and downstream (e.g., Rosa et al., 2011; Li and Venkatasubramanian, 2016; Torres-Acosta et al., 2016) strategies upon technical fluctuations or to evaluate stainless steel based, single-use based or hybrid facilities according to their likelihood of meeting desired cost of goods or project throughputs (Farid, Washbrook and Titchener-Hooker, 2005b). Examples of Monte Carlo simulation application in predicting facility fit issues are also given by Stonier et al. (2013) and Yang et al. (2014). Stonier et al. (2013) evaluated the facility fit for scale-up considering uncertainties in product titre, eluate volumes and step yields (triangular distributions applied). The likelihood of having product loss and of meeting facility demand was studied and the impact on cost of goods was estimated, proving that Monte Carlo

enables to identify risks and plan ahead on strategies to avoid facility constraints (Stonier et al., 2013). At the portfolio level, examples are shared by Rajapakse et al. (2005) and George and Farid (2008), who studied the impact of, not only technical, but also market uncertainties (e.g.: annual demand, drug price, market share) on the net present value using Monte Carlo simulations.

1.6.2.1.2 Multi-Criteria Decision Making

The evaluation of bioprocess viability can be extended through the application of multi-criteria decision making (MCDM) methods, where a set of alternatives is ranked in an order of preference according to pre-established attributes (Konstantinidis et al., 2012). The general structure of an MCDM process is depicted in **Figure 1.12** and there are three tasks which should be highlighted: the establishment of alternatives to consider, the evaluation criteria, and the relative importance of the different criteria (weights) (Pavan and Todeschini, 2009). In the biopharmaceutical industry, MCDM methods have been used to rank different manufacturing and portfolio approaches, aiding decision-makers in implementing sustainable processes. Additionally, as the industry is increasingly paying more attention to the environmental impact of bioprocesses, environmental metrics should also be incorporated as criteria. The ranking of different chromatography resins (e.g., Nfor et al., 2011; Stamatis et al., 2019), different upstream (e.g., Pollock, Ho and Farid, 2013) and downstream (e.g., Yang et al., 2017) techniques or conditions, or different process flowsheets (e.g., Farid and Jenkins, 2018) are some of the examples of MCDM application at the level of process design and facility fit. In capacity planning, build versus buy capacity sourcing strategies were also ranked according to certain business criteria, such as flexibility, location or manufacturing knowledge by George et al. (2007).

Table 1.7 and **Table 1.8** present a summary of research studies that used Monte Carlo simulations and multi-criteria decision making methodologies, highlighting the respective parameters in scope.

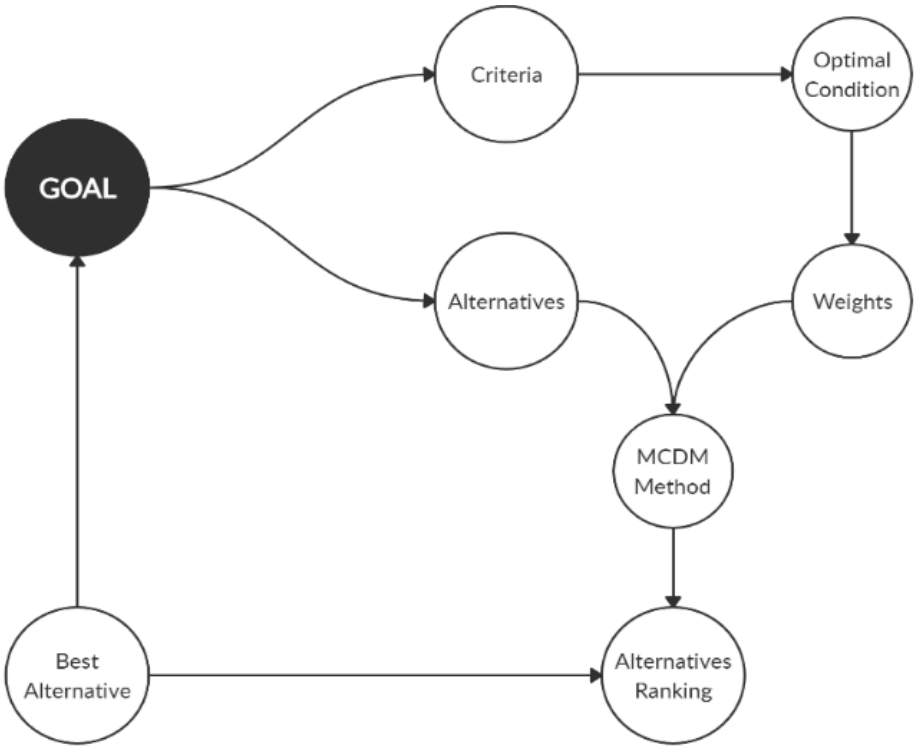


Figure 1.12 - Generic structure of multi-criteria decision-making tools. Adapted from (Pavan and Todeschini, 2009).

Table 1.7 - Literature overview on decision-support tools used on process synthesis and facility design models.

Case Study (Reference)	Monte Carlo Simulation			Multi-criteria decision making		
	Sources of Uncertainty		Metrics	Alternatives	Criteria	
SS versus SU facilities (Farid, Washbrook and Titchener-Hooker, 2005a)				• SS based • SU based • Hybrid	• Capital investment • Annual COG • Construction time • Project throughput	• Online control • Validation effort • Ease of scale-up • Suppliers reliance
SS versus SU facilities (Farid, Washbrook and Titchener-Hooker, 2005b)	• Fermentation titre • DSP Yield • Phase I demand • FCI	• Material Cost • WFI Cost • Turnaround time	• COG • Market Success • Project Throughput			
Pooling strategies in perfusion (Lim et al., 2005)	• Fermentation titer • DSP Yield • Turnaround time • Lang factor	• Media cost • WFI Cost • Operator Wage	• Project throughput • COG			
Perfusion versus batch processes (Lim et al., 2006)	• Fermentation titre • DSP yield • Lang factor Media cost	• CIP reagents cost • Reagents cost	• Annual output • COG • NPV			
Chromatography Design (Nfor et al., 2011)				• Different chromatography resins	• Purity • Yield • Concentration factor	• Productivity • Throughput • Cost
Continuous ATPS as capture step (Rosa et al, 2011)	• Protein A DBC • Protein A lifespan	• WFI cost • Waste/disposable cost	• Operating cost • Capital investment			
Feb-batch vs perfusion (ATF vs spin-filter) (Pollock, Ho and Farid, 2013)	• Scale of production • Fermentation titer	• Water consumption • Consumables	• Project Throughput • COG • Batch failures	• Fed-batch culture • Spin-filter perfusion • ATF perfusion	• COG • Initial capital expenditure • Water E-factor • Consumable E-factor • Batch-to-batch variability	• Ease of control • Operational flexibility • Ease of development • Ease of validation
Prediction of suboptimal facility fit (Stonier et al., 2013)	• Product Titer • Eluate Volumes	• Step Yields	• Product Mass Loss • COG • Batch duration • Processing time • Batch Cost			
Prediction of suboptimal facility fit (Yang, Farid and Thornhill, 2014)	• Product titre • Step yields	• Eluate volumes • Filter flux rates	• Product mass loss • Processing time			
Process Intensification through continuous mode (Walther et al., 2015)	• Technical transfer delays • Product failure	• Product demand	• NPV			
mAbs process development (Li and Venkatasubramanian, 2016)	• Protein A yield • Protein A loading	• IEX I/II yield • IEX I/II loading	• COG			
UF/DF conditions in mAbs manufacturing (Yang et al., 2017)				• Different formulation designs	• Viscosity Fed-batch culture	• Thermostability
ATPS and TFF as extraction strategies (Torres-Acosta et al., 2018)	• Extract titer • Recovery Yield	• Material Cost	• COG			
Bioprocess design of CAR-T cell therapies (Jenkins and Farid, 2018)	• Capital investment • COG per dose • Process control • Process containment	• Ease of scale-up • Ease of validation • Validation effort	• Overall performance aggregated score	• Different flowsheets	• Capital investment • COG per dose • Process control • Process containment	• Ease of scale-up • Ease of validation • Validation effort
HTPS in chromatography design (Stamatis et al., 2019)	• Load pH • Load conductivity • Load linear velocity	• Elution pH • Load Challenge • Elution linear velocity	• DBC • Purity • Yield • Elution Pool Volume	• Different chromatography resins	• HMW species removal • Purity change	• Yield • Productivity
Process Economics of AAVs (Lyle et al., 2023)	• Cell productivity • Cell density	• Centrifugation yield • AEX yield	• COG			

Table 1.8 - Literature overview on decision-support tools used on portfolio management and capacity models.

Case Study (Reference)	Monte Carlo Simulation		Multi-criteria decision making	
	Sources of Uncertainty	Metrics	Alternatives	Criteria
Portfolio management (Rajapakse, Titchener-Hooker and Farid, 2005)	• Market Share • Drug price • Development Time • Clinical Trial Time • Personnel • Building Delay • CMO Negotiation Time • Presence of a competitor • Mass per batch • Manufacturing Cost • Product Demand • CM Time	• NPV		
Capacity planning (George, Titchener-Hooker and Farid, 2007)			• Built plant • Partner with other company • Contract manufacturing (CMO) • Hybrid Partner/Build • Hybrid CMO/Build • NPV • Profits to sales • Profits to assets • Profits to equity • Sales to fixed assets • Profit to current assets • Sales to equity • Average inverted COGS • Profit to manufacturing personnel	• Qualitative productivity score • Location • Flexibility • Manufacturing knowledge • Current assets to current liabilities • Equity to liabilities • Assets to equity
Portfolio management (George and Farid, 2008)	• Annual demand • Compound annual growth rate • Target identification cost • Scale-up cost • Commercial preparation cost • Marketing Cost • FDA Review Cost • Clinical trial costs • Manufacturing Costs • Process Yields • Fermentation titer • Batch success • Ramp time to peak market • Decay time after market	• NPV		

1.6.3 Decision-support tools in continuous production of biopharmaceuticals

As biopharmaceutical companies have started the research and implementation of continuous manufacturing in their processes, the application of decision-support tools has been crucial on the overview and understanding of the best alternatives comparing to batch schemes. This chapter highlights some examples (**Table 1.8**) and conclusions obtained from applying process economics and other decision-support methodologies to relevant industrial case studies of continuous manufacturing.

As the primary focus of the biopharmaceutical industry on implementing continuous manufacturing has typically been on upstream processing, the comparison between perfusion technologies and fed-batch culture emerged as one of the early scenarios for applying computer-based tools in the simulation and assessment of continuous processes. Lim et al. (2006) described the importance of Monte Carlo simulation in bioprocess design and showed that, although the perfusion option had a higher NPV and required less capital investment under deterministic evaluation, the fed-batch option was found to be more robust when accounting for risks and uncertainties. Pollock et al. (2013) used sensitivity analysis and multi-criteria decision making to compare fed-batch with two perfusion strategies and concluded that the alternating tangential filtration (ATF) perfusion operation could offer economic advantages in cell culture; however, if environmental or operational feasibility were preferable over economic savings, fed-batch was the preferred strategy. Several options and conditions within perfusion cultivation, such as, different pooling strategies or media optimisation were also evaluated (e.g., Lim et al., 2005; Xu et al., 2017), showing relevant effects in production productivity. The impact on capacity planning compared to fed-batch has also been studied, indicating that perfusion processes could offer higher productivity and flexibility compared to fed-batch processes, but they also required more complex planning and management due to the continuous nature of the operation (e.g., Siganporia et al., 2014).

Regarding downstream processing, purification strategies have been a core focus when simulating continuous processes. The impact of (semi) continuous chromatography has been assessed by Pollock et al. (2013), who highlights the reduced costs offered by PCC in the early phase – “proof of concept” material generation. Xenopoulos (2015) also showed cost reductions higher than 20% and 30–60% at commercial and clinical scales, respectively, using several continuous DSP units integrated, with potential improvements as titre and scale increase. The integration of continuous upstream and downstream processing has also been simulated and the goal has been to understand the benefits that companies can have by integrating either hybrid (batch and continuous unit operations) or end-to-end continuous processes. Pollock et al. (2017) found that an integrated continuous strategy can be preferred for early phase production in small and medium-sized companies and that a hybrid strategy can be preferred in commercial production and in companies with large portfolios. Walther et al. (2015) used decision-support tools and Monte Carlo simulation to compare the net present value of integrated continuous schemes of mAb and non-mAb production and suggested savings of 55% on average using continuous manufacturing instead of conventional batch platforms. More recently, Mahal et al. (2021) demonstrated that single-use continuous facilities offered more substantial cost savings over stainless-steel batch at lower and medium scales (100-500 kg/year) compared to larger demands (>1000 kg/year), as the latter required parallel production trains and, thus, higher capital investments.

Although **Table 1.9** depicts the broad application of decision-support tools in continuous manufacture, it also reveals the limited research on crucial topics: the combination of cost and environmental outputs of batch and end-to-end continuous manufacturing processes, the analysis of end-to-end continuous manufacturing through different production flowsheets (e.g., column-free techniques comparing to standard column-based processes) and the application of Monte Carlo simulations to evaluate the robustness of different process under uncertainty were not found in literature altogether and are essential to create a comprehensive overview of end-to-end continuous manufacturing capabilities and trade-offs.

Table 1.9 - Literature overview on decision support tools applied in continuous biomanufacturing models. DES: discrete-event simulation; rec P: recombinant protein.

Case Study/Reference	Focus	Simulation	Decision-support tools	Metrics
Pooling strategies in perfusion (Lim et al., 2005)	USP rec P	Extend (DES)	• Process Economics • Monte Carlo Simulation	• Project throughput • COG
Perfusion versus batch processes (Lim et al., 2006)	USP rec P	Extend (DES)	• Process Economics • Monte Carlo Simulation	• COG • Capital investment • NPV
Feb-batch vs perfusion (ATF vs spin-filter) (Pollock, Ho and Farid, 2013)	USP mAb	Extend (DES)	• Process Economics • Monte Carlo Simulation • MCDM	• Project Throughput • COG • Batch failures • E-factor • Operational benefits
Media cost and productivity in perfusion vs fed-batch (Xu et al., 2017)	USP mAb	BioSolve	• Process Economics • (brief sensitivity analysis)	• COG
Capacity planning (Siganporia et al., 2014)	USP rec P	General Algebraic Modelling System	• Process Economics • Optimisation	• Facility utilisation • Inventory Cost • Inventory penalty cost • Variable cost • Fixed cost • Transportation cost • Waste cost • Backlog penalty cost • Facility investment • Retrofitting cost • License cost
Continuous ATPS as capture step (Rosa et al., 2011)	DSP mAb	Excel & SuperPro Designer	• Process Economics • Monte Carlo Simulation	• Operating cost • Capital investment
Semi-continuous affinity chromatography (Pollock et al., 2013)	DSP mAb	Extend (DES)	• Process Economics • Monte Carlo Simulation • MCDM	• Project Throughput • COG • Batch failures • E-factor • Operational benefits
Continuous precipitation (Hammerschmidt et al., 2014)	DSP mAb	BioSolve	• Process Economics • Monte Carlo Simulation	• COG
Continuous integrated DSP (Xenopoulos, 2015)	DSP mAb	BioSolve	• Process Economics • Monte Carlo Simulation	• COG
Integrated continuous production of recombinant proteins (Walther et al., 2015)	USP & DSP rec P	BioSolve	• Process Economics • Monte Carlo Simulation	• NPV
Batch vs continuous antibody production (Pollard et al., 2016)	USP & DSP mAb	BioSolve	• Process Economics • Monte Carlo Simulation	• Capital investment • COG • Facility utilisation • Net present cost
Batch vs continuous antibody production (Klutz et al., 2016)	USP & DSP mAb	Not disclosed	• Process Economics	• COG
Batch vs semi-continuous antibody production (Pollock et al., 2017)	USP & DSP mAb	Extend (DES)	• Process Economics • Monte Carlo Simulation • MCDM	• COG • E-factor
Batch vs continuous antibody production (Arnold et al., 2019)	USP & DSP mAb	BioSolve	• Process Economics	• COG
Batch vs continuous antibody production with different capture alternatives (Cataldo et al., 2020)	USP & DSP mAb	BioSolve	• Process Economics	• COG • Process mass intensity
Batch vs continuous antibody production (Mahal, Branton and Farid, 2021)	USP & DSP mAb	Python	• Process Economics	• COG

1.7 Aim and organisation

Existing research on biopharmaceutical manufacture and decision-support tools was reviewed in the previous sections. A special focus was placed on publications targeting mAbs, as the biological molecule of this thesis. Moreover, biomanufacturing in continuous mode was addressed and alternatives within upstream and downstream processing were discussed.

Through **Chapter 1**, one could infer that the effort of both academia and industry in tackling the challenges of biomanufacturing is clear. Whilst new technologies have been enabling the conversion of batch processes into faster, more flexible and productive continuous operations, decisional tools have been keeping up the pace of innovation by presenting economic evaluations of latest alternatives and their impact on facility capacities and cost of goods.

While protein A chromatography stands as a highly specific but expensive step in mAb manufacturing, the economic evaluation of end-to-end continuous processes with column-free options and the environmental impact of such changes in biopharmaceutical production have been scarcely approached by literature. Also, the benefits of enhanced control through advanced analytical technologies are rarely translated into tangible economic outputs. With the push for coupling continuous technologies with smaller, single-use based facilities, and with real-time control, there is a need for studying the consequent changes in costs and environmental footprint of new flowsheets.

The aim of this thesis is to develop a decisional tool that supports the evaluation of batch and continuous flowsheets with different processing alternatives and control systems. The simulation and optimisation of mAb manufacture will facilitate a more informed decision-making, which reconcile technical, economic and environmental feasibilities.

In **Chapter 2**, the decision-support tool structure and the manufacturing domain is described. The scope of the tool and requirements specifications are crossed with the capabilities of different software. The interface to build the process economics will also be selected. The modelling approach and key process models for batch and continuous unit operations are further addressed and the economic and environmental metrics are introduced.

The first chapter of results, **Chapter 3**, focus on the evaluation of mAb capture alternatives. Aqueous two-phase extraction (ATPE) and precipitation were selected as the most mature technologies from the column-free alternatives pool and with the highest technical potential to compete with protein A chromatography. Economic and environmental scores are derived from integrating ATPE and precipitation in the process economic model and the performance of these options is evaluated against protein A.

Chapter 4 presents the application of a life cycle assessment tool to estimate the product carbon footprint of different mAb flowsheets. The key contributors for the carbon emissions are identified to determine the best optimisation routes for carbon footprint reduction. The resulting emissions from each flowsheet before and after optimisation is then converted into common daily metrics to provide an easier view of the environmental impact associated with mAb manufacture.

In **Chapter 5**, the view of the biopharmaceutical sector on process analytical technologies (PAT) and enhanced control is assessed through a survey and interviews. The economic impact of state-of-the-art analytical technologies integrated in current and future mAb facilities is also evaluated via the decisional tool framework. Different degrees of process improvement and technology investment are addressed to simulate the cost savings and financial return of implementing PAT.

Chapter 6 presents the main conclusions of this thesis and suggests future research directions to expand the insights delivered by this work.

Chapter 2: Materials and methods

2.1 Introduction

The previous chapter highlighted the challenges faced by biopharmaceutical companies and their endeavours to develop more cost-effective products, which has prompted the exploration of continuous manufacturing. However, questions regarding the competitiveness of these innovative platforms from economic, environmental, and robustness perspectives persist. The importance of using predictive software to assess and optimise the technical and business performance of bioprocesses has also been highlighted, proving crucial for decision-makers when selecting strategies.

In this chapter, a decision-support tool designed to simulate and assess different production platforms will be described alongside with the manufacturing domain in scope. **Section 2.2** presents the required specifications for the tool and analyses the eligibility of different software to use. **Section 2.3** elaborates on the tool implementation and structure, including key features, parameters, and equations, while economic and environmental metrics are outlined in **Section 2.4**.

2.2 Scope of the tool

As described in **Section 1.5**, monoclonal antibodies are produced through mammalian cell culture fermentation and then recovered and purified via a series of downstream processing steps. Within each production platform several options can be taken, as the fermentation can be operated in batch, fed-batch or continuous perfusion mode and the DSP, even after deciding on the operation mode (batch, semi-continuous, continuous), can be designed by numerous different technologies with different configurations. Alongside with the selected unit operations, there are other ancillary activities, such as the cleaning of equipment or preparation of intermediate solutions that should also be considered. Moreover, the biopharmaceutical facility itself must be scaled according to the production demands for clinical trials or larger scale commercialisation and the scheduling of different operations must be optimised to minimise USP or DSP bottlenecks. As a result, it is vital to define the software

requirements to address this range of operations and needs and ultimately enable the calculation of operational, economic and environmental scores for different alternatives. The scope of the tool was built upon previous work from the Decisional Tools group at UCL (Farid, 2007; Pollock, Ho and Farid, 2013) and it is defined as follows:

- To enable the simulation of a biopharmaceutical facility to produce different products, process configurations, process performances and product demands in a campaign basis (e.g. 14 days fed-batch, 28 days perfusion) according to user-defined mass balance, sizing and costing equations;
- To enable the customisation/update of prices, equipment/ materials characteristics, and process parameters databases;
- To enable a production design in sizing mode, where the facility/equipment size is defined by a pre-set product demand, or in rating mode, where the facility/equipment size is fixed, and the production output is calculated;
- To track, record and distinguish input and output parameters/results from each step during the train of unit operations, including labor and equipment resources, raw materials consumption, and processing times;
- To evaluate and directly compare different manufacturing alternatives across designs (e.g., batch vs continuous) and technologies (e.g. chromatography vs precipitation) in terms of cost, environmental impact and robustness for different product demands;
- To capture different resource requirements between batch and continuous production modes in a dynamic environment;
- To enable different value distributions of input parameters and statistically evaluate the performance of different alternatives under uncertainty (e.g., impact of batch-to-batch titre variability, yield variability);
- To enable direct and rapid plotting of selected outputs across scenarios.

2.2.1 Software selection

Once the framework's scope and requirements were established, an analysis was conducted to determine the suitability of different bioprocess software or mathematical languages for modelling the decision-support tool. Based on the review presented in **Section 1.6** it was possible to ascertain which available interfaces could meet the project's needs. **Table 2.1** provides an overview of the tool prerequisites and assists in selecting the simulation package that encompasses most of the required capabilities.

Table 2.1 - Overview of bioprocess software and mathematical programming languages capabilities

	SuperPro Designer	**aspenONE**	**BioSolve**	**Programming Languages**
Mass Balances	✓	✓	✓	✓
In-process parameters/results recording	✓	✓	✓	✓
Sizing/rating mode	✓	✓	✓	✓
Cost analysis	✓	✓	✓	✓
Environmental analysis	✓	✓	✓	✓
Direct results visualisation across scenarios	✓	✓	✓	✓
Uncertainty analysis	×	×	×	✓
Dynamic modelling	×	×	×	✓
Fully customisable database	×	×	×	✓

While commercially available software like SuperPro Designer, aspenONE, or BioSolve offer many of the needed capabilities for constructing the tool, they seem to lack the capacity to incorporate dynamic and stochastic dimensions of modelling simultaneously, failing to meet the tool's design requirements.

The distinction between static and dynamic modelling determines whether systems can be represented over time. This distinction is particularly relevant when simulating continuous manufacturing. Static modelling (spreadsheet-derived) only captures bioprocesses at a specific moment, while dynamic modelling allows for the evolution and design of processes over time. In the current project, simulating tasks sequentially or in parallel and allocating resources must be done in a dynamic, time-dependent environment. Regarding deterministic versus stochastic simulations, although deterministic modelling is essential during the initial design of a biopharmaceutical facility, stochastic simulations provide valuable insights into the range of possible outcomes if input values (i.e., process parameters across unit operations) change, representing the inherent variability of large-scale production.

Due to their flexibility and user-defined structure, programming languages (such as C-Sharp, Python, etc.) can be employed to design discrete-event tools incorporating the envisioned dynamic and stochastic capabilities. Other available discrete-event simulators, such as ExtendSim® (Imagine That, California, USA) or ProModel® (ProModel Corporation, Utah, USA), also allow dynamic and stochastic modelling. However, they are not as customisable in terms of coding or database and often require linkage to external sources.

Among mathematical languages, Python has emerged as a prominent choice for scientific programming due to its speed, performance, and strong support as an open-source community-based language (Langtangen and Cai, 2008). Consequently, Python was selected to develop the dynamic stochastic decision-support tool, encompassing all the requirements mentioned earlier. The tool's architecture and implementation are detailed in the following section.

2.3 Tool implementation

In a manufacturing facility, key tasks and resources can be described in a hierarchical representation, where each higher-level activity is broken down into sub-tasks (Lim et al., 2004; Mustafa et al., 2004; Rajapakse, Titchener-Hooker and Farid, 2005). As illustrated in **Figure 2.1**, the levels in the hierarchy are modelled separately and built onto each other, increasing the complexity and consequent accuracy of the complete system.

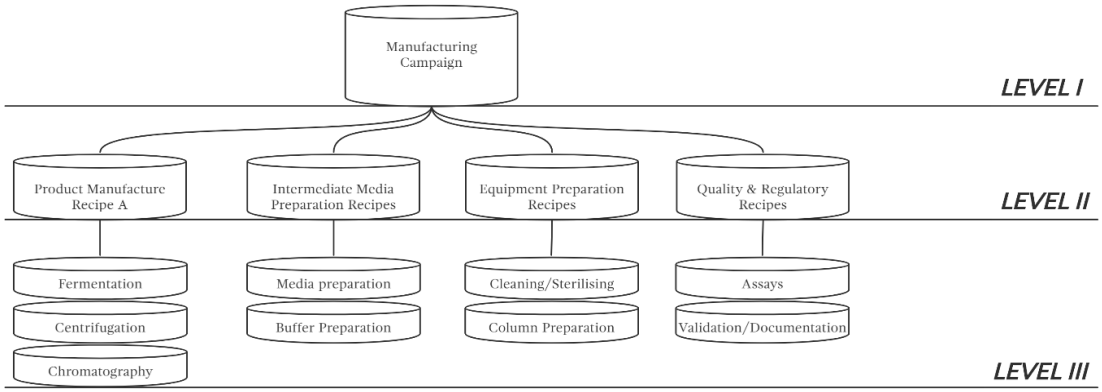


Figure 2.1 - Hierarchical decomposition of manufacturing tasks in a bioprocess. Sourced from Lim et al. (2004).

The model was developed through the object-oriented features of Python that allow the progressively design of a facility activity-based that pulls specific information from different classes. The translation of the hierarchical approach is achieved by having tasks which are described once and further used to create specific steps in the process as well as having functions which are written and then invoked as needed. Also, just as complex functions can be built from simple functions, large programs are built up from smaller subsystems that are documented and tested individually. These subsystems have well-defined input and output interfaces and can be used as libraries that enable to generate new programs using simple coding vocabulary. The decomposition of a complex bioprocess models in this manner decreases the extension of the model and improves the transparency and accuracy of the simulating tools.

2.3.1 **Modelling structure**

In the hierarchal approach adopted to model the required tool, different classes were implemented into the framework and are represented in **Figure 2.2**.

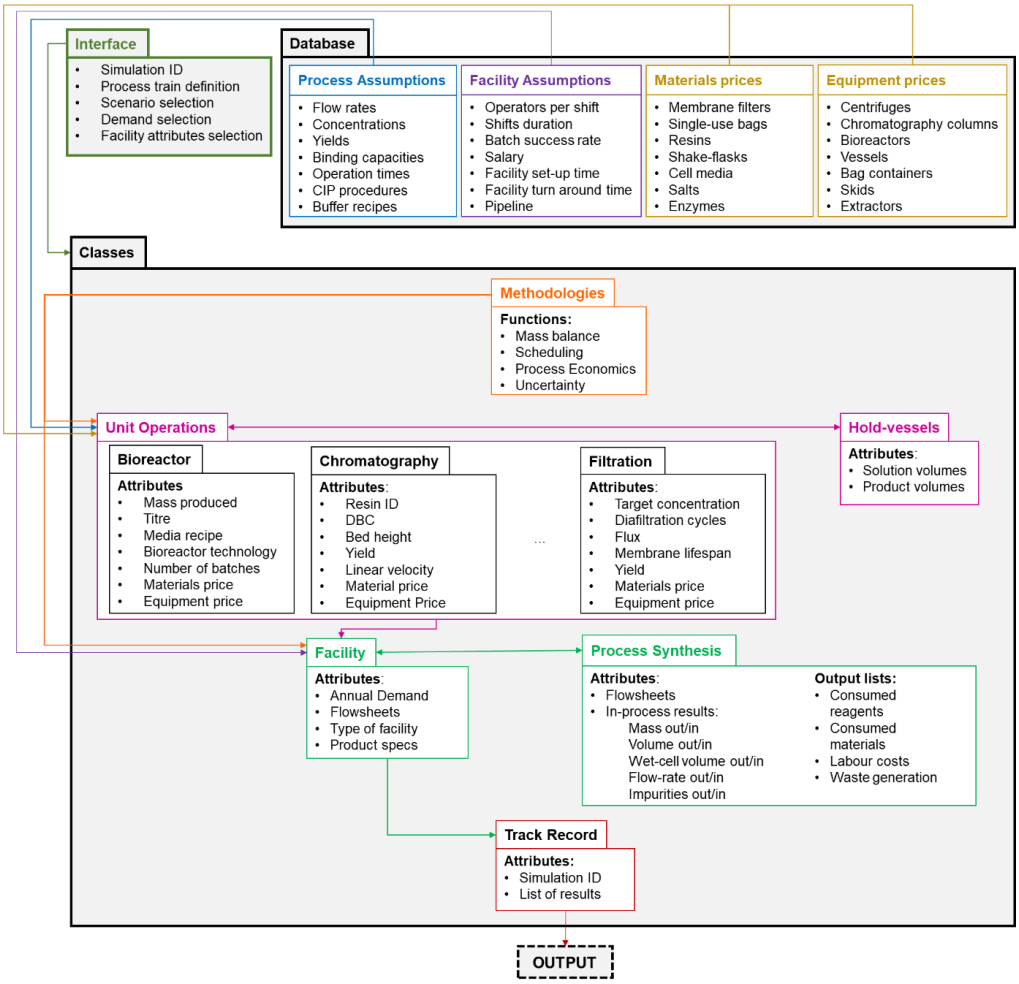


Figure 2.2 - Tool structure with breakdown of classes and respective attributes. The tool portrays the database, assumptions and classes needed to the different case studies (i.e., batch, continuous, column-based, column-free).

In an object-oriented manner, each class, with its own functions and attributes, can be affiliated with others, generating outputs that correspond to input parameters of the linked object. In the present framework, there are two types of classes:

1. The ones corresponding to unit operations in the bioprocess train (e.g., centrifugation, chromatography), which include specific process and sizing equation for that technology, and

2. The ones comprising support functions and tasks that allow the connection between all the elements in the model and computation and recording of intermediate/final results (e.g., facility, process synthesis, track record).

Before modelling the desired scenarios for the project, discussions with academic and industrial partners and vendors (e.g., IST-Lisbon, AstraZeneca, CPI, Sartorius, PALL, ThermoFisher, and others) were held to gain understanding and gather process/facility assumptions and equipment/material specifications/prices, so a database of values to input into the process economics model could be created. Throughout time, this database was updated and extended, so the model output would represent realistic production schemes as much as possible.

Regarding the modelling task, the definition of a certain modelling scenario started by creating a list of unit operations in sequence in the interface upon which the model would run. The different flowsheets considered for each case-study are presented in **Chapter 3**. Then, the *Facility* class, which recognises the user-defined process train (flowsheet) and annual product demand for which the process must be designed (if in sizing mode), is associated with the *Process Synthesis* class, where the model runs through each unit operation in the list, triggering the calculation of outputs associated to each step. The procedure begins by calculating the mass of product needed after fermentation based on the annual product demand and on the intermediate step yields taken from the database or interface. In the *Bioreactor* class, for instance, the size and number of bioreactors to achieve the desired product throughput are calculated through mass balances (from *Methodologies* class) and sizing equations, according to specific parameters from the database (e.g., product titre after fermentation). In each unit operation class (e.g. *Bioreactor*, *Chromatography*, *Filtration*, etc.), most of the process assumptions are taken from the database and the calculation of process outputs is done through the application of functions defined in the *Methodologies* class. The process economic equations integrated in *Methodologies* make also use of the facility assumptions and prices gathered in the database and are described in **Section 2.4** and in the **Appendix A1**. Due to the existence of numerous secondary

tanks for solution preparation and product hold, the set-up of these vessels and calculation of the respective CIP buffer volumes required for cleaning, water for injection and process water is done in the *Hold-vessels* class. For each step in the process train, the outputs are recorded in lists through the *Process Synthesis* class, where also the in-process parameters, such as mass, volume or flowrate out of one unit are loaded as attributes of the next one. The lists from the *Process Synthesis* are finally read in the *Facility* class, which computes the overall economic and environmental metrics of the process. All the intermediate and final results are exported to a file through the *Track Record* class.

In the present simulation tool designed in Python, one can run several flowsheets and compute results for several product demands at once, increasing the speed of analysis and comparison of different scenarios. Also, in the running interface assembled, it is possible to automatically plot of the obtained results.

Figure 2.3 provides a comprehensive overview of the connections between inputs, tools, and outputs.

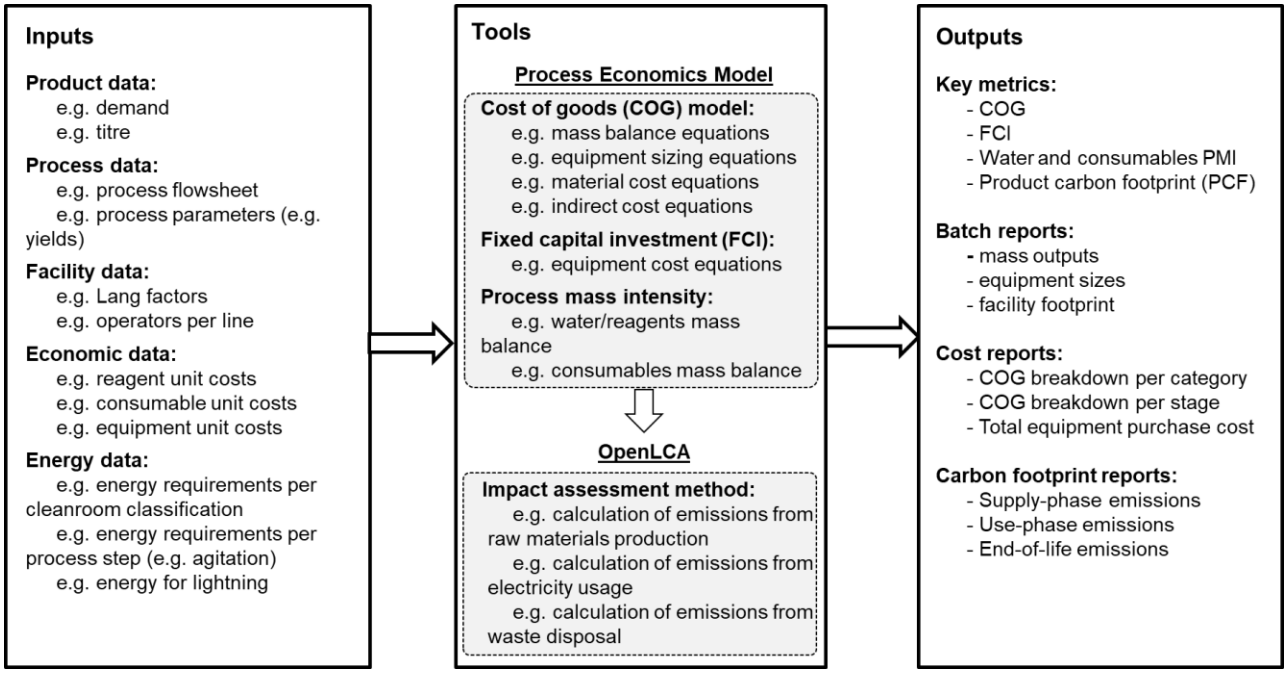


Figure 2.3 – Decisional tool framework, with examples of inputs, tool calculations and outputs.

2.3.2 **Modelling of unit operations**

In this section, the process models corresponding to the different unit operations in a desired process train are generally described. For each operation, designs in batch and continuous are modelled within the class. The fundamental assumption adopted between continuous units is considering that the flowrate into a step is equal to the flowrate out of the previous one. In cases in which this set-up is not possible (due to capacity or time – scheduling – constraints), an accumulation of product in hold-tanks is recognised and the ancillary vessels are created in the *Hold-vessels* class.

While mass stoichiometry models or detailed mass transfer equations can be used to design bioprocesses and calculate the output streams composition, these often lead to an extra level of complexity whose advantage is poorly represented in the overall facility design due to the inherent variability of process economics models. Therefore, *short-cut models* based on empirical correlations or parameters found in the literature are herein used to determine the size, number and other equipment characteristics, determine processing times and compute other outputs from each unit operation. As aqueous two-phase extraction and precipitation are the main unit operations in which this thesis is focused on, the mass balance equations are showed in the next chapter. All the equations involving the other used upstream and downstream processing techniques can be found in the **Appendix A1**.

2.3.2.1 Aqueous two-phase extraction

Aqueous two-phase extraction (ATPE) makes use of liquids with different physicochemical properties (special attention given to density) to extract the desired product into a phase, while most of impurities (DNA, HCP, and cell debris) migrate to another phase or stay in the interface of liquids.

Most of ATPE systems involve the mixture of the following components:

- Polyethylene-glycol (PEG), that will be the main compound of the phase to which mAbs will migrate (top phase).

- A salt, such as phosphate or citrate, or another polymer with different density from PEG, such as dextran, which will represent most of the impurities-rich phase (bottom phase).
- NaCl, which partitions evenly between both phases. On the one hand, it is used to increase the ionic strength of the bottom phase, reducing protein solubility. On the other, negatively charged chloride ions provide an increased electrostatic attraction of positively charged monoclonal antibodies to the top phase.
- Crude feedstock from fermentation, or harvest cell culture fluid (HCCF) (including cells and other solid particles in the batch scenario), whose components will be segregated into the different phases.

ATPE optimisation is achieved by finding the relative percentages of these four constituents (and “make-up” water) which lead to the best mAb recovery yield. In the *short-cut model* adopted, the percentages of PEG, salt and NaCl are based on literature and will be fixed, while the ratio of crude feedstock, as the driving force of the ATPE system sizing, may be optimised. The present project makes use of ATPE as an alternative capture step of protein A chromatography; thus, the unit operation is placed directly after cell culture.

In continuous ATPE, the aqueous two-phase extraction takes place in a glass column and the operation occurs countercurrently, where the top PEG-rich phase is continuously fed at the bottom of the column, while the bottom salt-rich phase is fed at the top of the column (Rosa et al., 2012). The product is recovered from the top PEG-rich phase.

The design of the glass column (or extractor), i.e. height and diameter, is, at a first stage, fixed. The flowrate out of the perfusion bioreactor will then allow the calculation of the total flowrate into the extractor (equation 2.1) and the consequent linear flowrate and residence time (equation 2.2 and 2.3). After, if the residence time achieved is lower or higher than the limits established, the diameter of the extractor is redefined according to the minimum or maximum residence times allowed (equation 2.4), otherwise, the initial extractor design is kept.

$$FR_{in\ total} = \frac{FR_{out\ perfusion}}{\mathrm{HCCF}} \quad (2.1)$$

$$LFR_{in\ total} = \frac{FR_{in\ total} \times 4000}{\pi \times D_{extractor}{}^{2}} \quad (2.2)$$

$$RT = \frac{H_{extractor}}{LFR_{in\ total}} \quad (2.3)$$

$$D_{extractor\ corrected} = \sqrt{\frac{FR_{in\ total} \times 4000 \times RT_{max/min}}{\pi \times H}} \quad (2.4)$$

Where $FR_{in\ total}$: Flow-rate of PEG, salt, NaCl and crude feedstock loaded to the extractor (L/h)

$LFR_{in\ total}$: Linear total flow rate (cm/h)

$D_{extractor}$: Initial assumed extractor diameter (cm)

RT: Residence time (h)

$H_{extractor}$: Extractor height (cm)

$D_{extractor\ corrected}$: Calculated extractor diameter based on assumed residence time (cm)

$RT_{max/min}$: Assumed maximum or minimum residence time (h)

The total ATPE volume will be calculated through equation 2.5.

$$WV_{ATPE} = \frac{V_{in}}{HCCF} \quad (2.5)$$

Where V_{in}: Perfusion volume (BWV) from cell culture (L)

WV_{ATPE} : ATPE volume (L)

HCCF : Ratio of harvested cell culture fluid in the ATPE system

The quantities of PEG, salt and NaCl are computed based on the percentages of each component defined in the literature, which are multiplied by the total extraction volume. Compensation water may be introduced to make up for the total volume.

The product stream that will be sent to the next unit operation corresponds to the top PEG-rich phase, whose volume is determined through the ratio between

the top and bottom phases (equation 2.6). This ratio is taken from literature and it is specific for each PEG/salt initial composition in the system (assuming a similar density and product concentration of the feedstock used in the literature). **Figure 2.4** shows a generic binodal curve and tie lines which represent the ATPE phases' diagram. PEG and salt mixtures below the bimodal curve form monophasic systems, while mixtures above will lead to two-phase regions. Any ATPE system prepared with the PEG/salt compositions along the dotted tie line will result in the same top and bottom phase conditions; however, for each starting PEG/salt concentrations, different volume ratios between the two phases will be obtained.

$$V_{out} = \frac{WV_{ATPE}}{\text{ratio}_{\frac{top}{bottom}}} \tag{2.6}$$

Where $ratio_{top/bottom}$: Volume ratio between top and bottom phases

As the extraction led to a dilution of the stream, a concentration step (single-pass filtration) was included afterwards to concentrate the product phase. A subsequent diafiltration step was added for buffer exchange and removal of the PEG from the system before the viral inactivation.

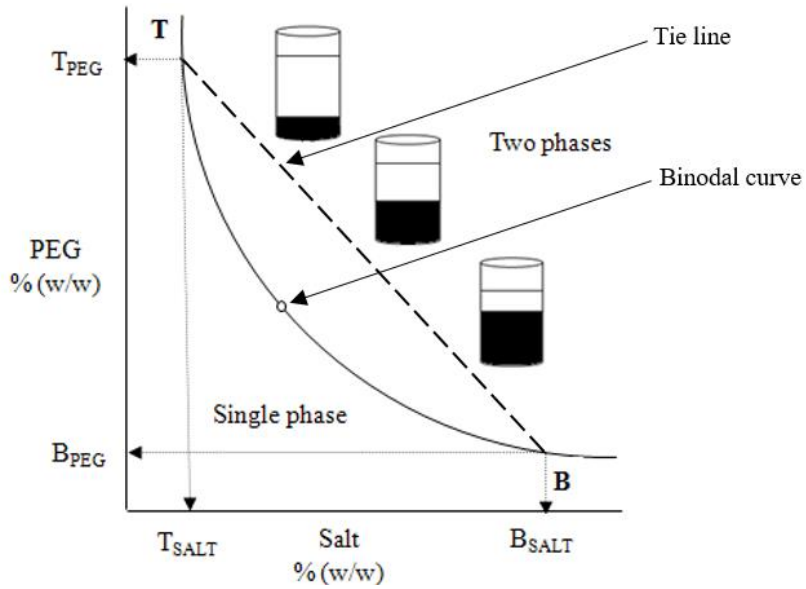


Figure 2.4 - Generic ATPE phase diagram. T_{PEG}: PEG composition in top phase, B_{PEG}: PEG composition in bottom phase, T_{SALT}: Salt (or other polymer) composition in top phase, B_{SALT}: Salt (or other polymer) composition in bottom phase. Sourced from Iqbal et al. (2016).

2.3.2.2 Precipitation

The principle behind precipitation (PP) lies in reducing the solubility of target molecules so they come out of solution in form of insoluble precipitates and can be separated. This technique can be used either to precipitate the product or impurities and efforts have been made to increase the selectivity of the process and increase recovery yields.

The PP system will then comprise:

- PEG, which increases the viscosity of the system and acts as a volume exclusion agent to enhance mAb precipitation;
- Zinc chloride, or other bridging salt, that cross-links to protein molecules in a flocculent-like manner and helps neutralising surface charges and precipitating the antibodies;
- Cell culture broth after cell removal, which contains the target product precipitate.

The harvested cell culture fluid (HCCF) and zinc are continuously fed to a static mixer, where product precipitation occurs inline. A second static mixer was placed in series, to which PEG is added to promote the growth of precipitates (Li et al., 2019).

In continuous precipitation, after discussion with experts, the static mixers described by Li et al. (2019) were designed as plastic tubes placed between the single-pass tangential flow filtration pump and the filtration membranes, which do not require any additional operating system. The sizing of the tubing is performed by fixing the length and residence time described in the publication and by computing the desired diameter based on the new flowrate (equation 2.7).

$$D_{tubular\ reactor} = \sqrt{\frac{FR_{in\ total} \times 4000 \times RT}{\pi \times L}} \qquad (2.7)$$

Where $FR_{in\ total}$: Flow-rate of PEG, zinc chloride and HCCF loaded to the tubular reactor (L/h)

$D_{tubular\ reactor}$: Initial assumed extractor diameter (cm)

RT: Residence time (h)

L: Length of the tubular reactor (cm)

The precipitates were then concentrated, washed and re-concentrated in a single continuous filtration unit to decrease the number of equipment required. After an in-line resolubilisation in another static mixer, a depth filtration was also included to avoid solid particles entering the remaining DSP. Before the viral inactivation, an extra concentration step was modelled to achieve the target concentration.

2.4 Cost and environmental models

The key performance indicators that will allow the evaluation and comparison of different scenarios are divided into 1) economic and 2) environmental metrics. This section will describe the procedures adopted for the calculation of the Fixed Capital Investment (FCI) and Cost of Goods (COG), as economic metrics, and of water and consumables PMI and PCF, as environmental metrics.

2.4.1 Fixed capital investment

The fixed capital investment (FCI) can be calculated through the product between the total equipment purchase cost and a cost factor which accounts for items such as piping, instrumentation, electrical work, site preparation, design, engineering and contract manufacturing fees. This simpler technique of calculating the FCI using a factorial method was originally suggested by Lang (1948) and the factors, called Lang factors, will depend on the type of facility in case. For biopharmaceutical facilities, these factors may vary between 3.3 and 8.1 (Farid, 2007). The present project assumes different Lang factors for stainless-steel based or single-use facilities, with values of 8.1 and 4.7, respectively. The calculation is described by equation 2.8.

$$FCI = L \times \sum Cost_{Equipment} \tag{2.8}$$

Where FCI: Fixed capital investment ($)

L: Lang factor

$Cost_{Equipment}$: Equipment purchase cost ($)

The equipment needed for each unit operation (i.e. specific hardware and ancillary solution and product tanks) is sized according to the scale required to respond to a defined annual product demand. As it is not always possible to obtain the equipment prices across all needed sizes, another factorial method (six-tenths rule) was used to relate the cost of equipment with its calculated size based on a known price found for another dimension (equation 2.9). The exponential scaling coefficient (c) is specific for different types of equipment and it is typically lower than 1, since the purchase cost is not linearly scalable with the equipment size.

$$Cost_{new} = Cost_{base} \times \left(\frac{Size_{new}}{Size_{base}}\right)^{c} \quad (2.9)$$

Where $Cost_{new}$: Cost of equipment with calculated size ($)

$Cost_{base}$: Cost known of equipment from same type and known size ($)

$Size_{new}$: Required equipment size (m, m^2, L, L/h, etc.)

$Size_{base}$: Size of equipment whose cost is known (m, m^2, L, L/h, etc.)

c: Exponential scaling coefficient (equipment dependent)

2.4.2 Cost of goods

The term Cost of Goods (COG) usually comprises 1) indirect and 2) direct manufacturing costs.

The indirect costs can be calculated through the fixed capital investment and include the depreciation of equipment, cost of general utilities, cost of maintaining and insuring the production facility and local taxes. The term of general utilities accounts for facility running cost, such as the HVAC systems used in the clean-rooms and depends on the facility size, which is calculated based on a function suggested by George (2008).

The direct costs are variable costs and include the expenditures in reagents, consumables, quality control materials and labour, depending on the amount of product manufactured. Reagents costs include all buffers (including cleaning buffers), media and water for injection required, while the consumables costs

encompass the cost of chromatography resins, pre-packed columns, membrane filters and all single-use materials. Quality control materials account for the reagents and consumables used in quality control tests of produced batches. Labour costs represent the cost of direct labour and the cost of additional supervision, management and QCQA (quality control and quality assurance) personnel. The direct labour costs are estimated based on a fixed annual salary and yearly facility utilisation, while the extra personnel expenses are derived based on this value.

The detailed breakdown of the COG calculation is presented in **Table 2.2**.

Table 2.2 - Breakdown of cost of goods for a biomanufacturing facility.

Fixed capital investment, FCI ($)	$FCI = L \times \sum C_{Equipment}$
Depreciation of equipment, C_{Depr} ($)	$FCI/t_{project}$
General utilities, C_{GenUt} ($)	$F_{size} \times F_{cost}$
Maintenance, C_{Mtn} ($)	$0.1 \times FCI$
Insurance, C_{Ins} ($)	$0.01 \times FCI$
Local taxes, C_{LocT} ($)	$0.02 \times FCI$
Indirect costs, $C_{indirect}$ ($)	$C_{Depr} + C_{GenUt} + C_{Mtn} + C_{Ins} + C_{LocT}$
Total labor costs, C_{labor} ($)	$C_{labor\ D} + C_{labor\ S} + C_{labor\ M} + C_{labor\ QCQA}$
Direct labor costs, $C_{labor\ D}$ ($)	$N_{operators} \times Salary \times u$
Supervisors' costs, $C_{labor\ S}$ ($)	$0.2 \times C_{labor\ D}$
Management costs, $C_{labor\ M}$($)	$C_{labor\ D}$
QCQA staff costs, $C_{labor\ QCQA}$ ($)	$C_{labor\ D}$
Facility utilisation, u (%)	$t_{campaign}/365$
Number of operators, $N_{operators}$	$N_{opUSP} + N_{opDSP}$
Number of USP operators, N_{opUSP}	$Shifts_{USP/day} \times N_{opUSP/shift} \times \frac{N_{USP\ trains}}{Train_{USPteam}} \times P_{trains}$
Number of DSP operators, N_{opDSP}	$Shifts_{\frac{DSP}{day}} \times N_{\frac{opDSP}{shift}} \times \frac{N_{USP\ trains}}{Train_{DSPteam}} \times P_{trains}$
Materials costs from QC tests, $C_{QC\ materials}$ ($)	$N_{QC\ batches} \times Price_{QC\ materials}$
Direct costs, C_{direct} ($)	$C_{reagents} + C_{consumables} + C_{QC\ materials} + C_{labor}$
Cost of goods per gram, COG/g ($/g)	$(C_{indirect} + C_{direct})/\ Demand$

Note:
L: Lang factor
$Cost_{Equipment}$: Equipment purchase cost ($)
$t_{project}$: Time of project (assumed 10 years)
F_{size}: Facility size (m^2)
F_{cost}: Monetary units per sq. meters (assumed 525 $/m^2)
$t_{campaign}$: Campaign running time (days)
$Demand$: Annual product demand (kg or g)
$Shifts_{USP/day\ or\ DSP/day}$: Shifts of USP/DSP per day
$N_{op/shift}$: Number of USP/DSP operators per shift
$N_{USP/DSP\ trains}$: Number of USP/DSP trains
$Train_{USP/DSPteam}$: Number of USP/DSP trains per team
P_{trains}: Product parallel trains
$Price_{materials}$: price of materials used in QC test ($)
$N_{QC\ batches}$: Number of cycles of final product out

2.4.3 **Environmental metrics**

2.4.3.1 Process Mass Intensity

The environmental burden caused by running a biomanufacturing facility will be evaluated through the water and disposables consumption. Process mass intensities (PMIs) will be calculated for each scenario across different product throughputs and, along with the economic metrics, will help comparing alternatives for the production of monoclonal antibodies.

The mass of water is determined by summing up the quantities of all liquid reagents consumed in the process. On the other hand, the mass of disposables is estimated based on the weights of all single-use materials (**Table 2.3**). A wide range of materials weights was collected for different dimensions; however, when the required size is not part of the gathered list, the weight of the consumable with the closest size is assumed. Equations 2.10 and 2.11 present the calculation of the water PMI and consumables PMI, respectively.

$$Water\ PMI = \frac{m_{water}}{Demand} \tag{2.10}$$

$$Consumables\ PMI = \frac{m_{disposables}}{Demand} \tag{2.11}$$

Where m_{water}: Quantity of water consumed per year (kg)

$m_{disposables}$: Mass of disposables consumed per year (kg)

$Demand$: Annual product demand (kg)

Table 2.3 - Weights of consumables used for the PMI calculation.

Material	**Example of weight & scale**
SU bag	7 Kg (2000 L)
Depth filter	5 Kg (2.5 m^2)
Virus removal filter	4 Kg (1 m^2)
Inline concentration/diafiltration (ILC/ILD) membrane	3 Kg (2.5 m^2)
UFDF membrane	2 Kg (1 m^2)

2.4.3.2 Life Cycle Assessment (LCA)

To perform the Life Cycle Assessment and compute the Product Carbon Footprint (PCF) associated to different flowsheets, OpenLCA was used. OpenLCA is a widely used open-source software tool for life cycle assessment (LCA) and sustainability analysis. It is typically used in conjunction with life cycle inventory databases, such as EcoInvent, which contain data on the environmental inputs and outputs associated with various processes and activities.

The workflow followed in OpenLCA was:

1. Creating processes

   Defining processes, which represented the stages of mAb life cycle (e.g., raw material extraction, manufacturing, use, disposal).

2. Adding flows

   Within each process (i.e., unit operation), the material and energy flows that go in and out of that process were added. Flows represented the inputs and outputs of the system.

3. Linking processes

   Processes were linked to show the flow of materials and energy between them.

4. Selecting methodologies

   Choosing the appropriate impact assessment methodology that aligned with the project goals (ReCiPe).

5. Running the analysis

   Quantifying the environmental impacts of each process in terms of selected impact categories (climate change).

6. Interpreting and presenting Results

   OpenLCA provided visualisation tools to help extract and understand the results.

2.4.3.2.1 Global Warming Potential (GWP) and Product Carbon Footprint (PCF)

Global Warming Potential (GWP) is a fundamental aspect of Life Cycle Assessment (LCA) used to assess the impact of greenhouse gas emissions on global warming. The GWP calculation is based on the concept of equivalency, which compares the warming potential of different greenhouse gases to that of carbon dioxide (CO2) over a specific time horizon.

The GWP represents a relative index that measures the potential of a greenhouse gas (GHG) to trap heat in the atmosphere compared to CO2. It is expressed as a factor that indicates how many times more effective a particular gas is at warming the atmosphere than an equivalent mass of CO2. GWP takes into account the radiative forcing effects of each gas and the time it remains in the atmosphere.

$$GWP = \Sigma\ (E \times GWP_{factor}) \qquad (2.12)$$

Where E: Quantity of a specific greenhouse gas emitted during a process, product life cycle, or activity (CO_2-eq).

GWP_{factor}: Global Warming Potential of the greenhouse gas in question, considering a specific time horizon.

The GWP factors are determined based on scientific assessments and are provided for various time horizons, usually 20, 100, and 500 years. The choice of time horizon depends on the intended focus of the assessment. The most common time horizon is 100 years, as it provides a balanced view of both short-term and longer-term impacts.

Product Carbon Footprint (PCF) is specifically concentrated on GWP as environmental impact category and it is directly calculated on the LCA platform used (openLCA). According to BioPhorum's Roadmap to Sustainability, there is an increasing demand for product transparency with respect to sustainability and metrics harmonisation, thus, PCF is a valuable tool, especially when

conducting a full Life Cycle Assessment (LCA) is resources and time consuming (BioPhorum, 2023).

2.4.4 Multi-criteria decision making (MCDM) methodology

The MCDM technique incorporated in the decisional tool was based on the weighted sum method and was designed to provide an overall measure of attractiveness for each flowsheet that reconciled economic, environmental and operational criteria (Pollock, Ho and Farid, 2013; Pollock et al., 2017). The economic (COG and FCI) and environmental (water and consumables process mass intensities (PMI)) ratings (x_{ij}) were directly obtained from the process economics model. The operational criteria identified for the analysis were robustness, ease of validation, ease of installation, ease of scale-up and ease of operation. The relative rating values of each flowsheet at each operational criteria (x_{ij}) and the rank of importance of each criterion amongst all operational criteria (E_i) were gathered from a survey questionnaire sent to industry and academic experts on the field. The criteria expressing economic (COG and FCI) and environmental feasibilities (water PMI and consumables PMI) were ranked equally within each criteria category based on personal communication with industrial partners. All rating values were standardised to a common dimensionless scale (r_{ij}) between 0 and 100 according to equation 2.13.

$$r_{ij} = \frac{x_{ij-}x_{iWorst}}{x_{iBest-}x_{iWorst}} \times 100 \qquad (2.13)$$

The weight of each criterion, E_i, was based on the rank of importance (most important weighs the most) and was then normalised as w_i according to equation 2.14.

$$w_i = \frac{E_i}{\sum_{i=1} E_i} \text{ , where } Ei = a, a-1, \dots, 1 \text{ for } rank = 1, 2, \dots, a \qquad (2.14)$$

The weighted score of each flowsheet for each criterion, y_{jk}, (i.e., economic, operational and environmental) was derived using equation 2.15.

$$y_{jk} = \sum_{i=1}^{n} r_{ij} \times w_i \tag{2.15}$$

The overall aggregated score, S_j, was computed according to equation 2.16. The ratios of importance of each criteria category (R_k) enabled the priorities of the economic, environmental and operational criteria to be altered based on user preferences, where the sum of the R_k values was equal to 1 ($R_{eco} + R_{env} + R_{op} = 1$).

$$S_j = \sum_{k=1}^{n} y_{jk} \times R_k = y_{j,eco} \times R_{eco} + y_{j,env} \times R_{env} + y_{j,op} \times R_{op} \tag{2.16}$$

Where j: $alternative$ $(Conti - ProA, Conti - ATPE, Conti - PP)$

k: $criteria\ category$ $(economic, environmental, operational)$

i: $criterion$ $(COG, FCI, PMI, robustness, ease\ of validation, etc)$

r_{ij}: $standardised\ rating\ of\ alternative\ j\ in\ subcriterion\ i$

x_{ij}: $rating\ value\ of\ alternative\ j\ in\ subcriterion\ i$

$x_{iWorst/Best}$: $Worst/Best\ rating\ value\ for\ subcriteron\ i\ among\ all\ alternatives$

w_i: $normalised\ weight\ of\ criterion\ i\ in\ category\ k$

E_i: $weight\ of\ criterion\ i\ in\ category\ k\ (based\ on\ rank)$

a: $number\ of\ rankings$

y_{jk}: $weighted\ score\ of\ each\ alternative\ j\ in\ category\ k$

S_j: $overall\ aggregated\ score\ of\ alternative\ j$

R_k: $ratio\ of\ importance\ of\ criteria\ k$

Chapter 3: Evaluating end-to-end continuous antibody manufacture with column-free capture alternatives from economic, environmental and robustness perspectives

3.1 Introduction

Across the years, a highly standardised platform has been shared for the production and purification of monoclonal antibodies. In most instances, this platform has been including protein A affinity chromatography as primary mAb capture step, due to the high binding levels and purities obtained; however, disadvantages of using such technique include the high costs associated to the resin, ligand leaching and poor stability at high pH, which have elevated the need of investigating new alternatives that allow to overcome such challenges and develop a renovated, protein A-free capture step.

The technical potential of column-free alternatives for capture based on either aqueous two-phase extraction (ATPE) or precipitation (PP) has been discussed in **Chapter 1**. Nevertheless, the economic evaluation of these technologies when integrated in a full production platform has been scarcely reported, leaving questions on the feasibility of these operations compared to protein A based chromatography.

In this chapter, the impact of integrating ATPE or PP is presented at economic, environmental and robustness levels using the tool developed in **Chapter 2**. Moreover, the study includes the modelling of batch and continuous production schemes with protein A capture, broadening the evaluation and providing extended insights on how the simulation framework can be used in different scenarios.

Section 3.2 presents the flowsheets, key assumptions and process parameter values used in the case studies, while **Section 3.3** deals with the results and discussion. **Section 3.4** summarises the main conclusions on the comparison between scenarios.

3.2 Methods

The tool developed in **Chapter 2** was used to model and evaluate commercial mAb facilities using different production schemes across different annually required product quantities. The case-study explored three antibody capture technologies: protein A affinity chromatography, aqueous two-phase extraction (ATPE) and mAb precipitation. While Batch-ProA depicted a typical fed-batch process, the continuous flowsheets integrated a perfusion bioreactor that enabled the retention of the cells inside the bioreactor and, therefore, did not require centrifugation and depth filtration as primary recovery steps before mAb capture. The simulation of the batch and continuous flowsheets integrating protein A chromatography was described by Mahal, Branton and Farid (2021). In Conti-ATPE and Conti-PP, only the capture stage was re-designed, thus, the process modelling from the viral inactivation to the final inline diafiltration was kept. The description of the unit operation and respective sizing equations for Conti-ATPE and Conti-PP were described in **Sections 2.3.2.1** and **Section 2.3.2.2**, respectively.

All batch and continuous process trains were designed to produce 100 to 1000 kg per year. The cell culture size was calculated based on the annual demand required and on the overall yields computed for the DSP train in each flowsheet. Bioreactor sizes were adjusted based on vendor constraints with a maximum size of 20,000L for stainless steel bioreactors and 2000L for single-use bioreactors. The single-use based continuous production flowsheets (Conti-ProA, Conti-ATPE and Conti-PP) were compared with the reference batch stainless-steel facility type with protein A chromatography as capture step (Batch-ProA). For Batch-ProA, the equipment sizing was based on the mass entering each unit operation and all steps were carried out sequentially. For the continuous options, the sizing was based on the outlet flowrate of the previous unit operation in the process train and the end-to-end continuous process was achieved through keeping the product outlet and inlet flows between units constant to avoid surge vessels or hold-times. When sizing the continuous ATPE glass column, the height was kept constant, whilst for continuous precipitation the length of the static mixers (Li et al., 2019) was kept constant.

The cost of goods and PMI metrics were computed according to the methodology described in **Section 2.4**. Following a deterministic analysis based on values from literature, previous group projects, and discussions with academic and industrial partners, a sensitivity analysis was conducted to predict the maximum variation in cost of goods resulting from broad changes in process parameters (worst vs best outcomes). After the sensitivity analysis, Monte Carlo simulations were employed to comprehend and compare the robustness upon process variability of ATPE and precipitation with protein A chromatography. For both analyses, the range of values for ATPE input parameters was deliberated with IST-Lisbon, while the precipitation technique and inputs were reviewed with BOKU and AstraZeneca. This approach aimed to capture both the present and future capabilities of these technologies.

The integration of economic, environmental and operational metrics was subsequently done through multi-criteria decision-making (MCDM), which reconciled quantitative outputs from the process economics model and qualitative scores obtained for each technology from an industrial survey. A final target analysis was generated by changing relevant process parameters and analysing the combination of inputs that would turn column-free options 15% cheaper the current ProA chromatography in continuous mAb manufacturing.

3.2.1 Flowsheets & key assumptions

As aforementioned, the goal of this study was to compare different capture alternatives and production running modes, which was fundamentally translated in modelling different mAb manufacturing flowsheets. **Figure 3.1** shows the different process sequences studied within the project and **Table 3.1** summarises the key input assumptions for each flowsheet. These inputs were integrated in the mass balance and sizing equations described in **Chapter 2**.

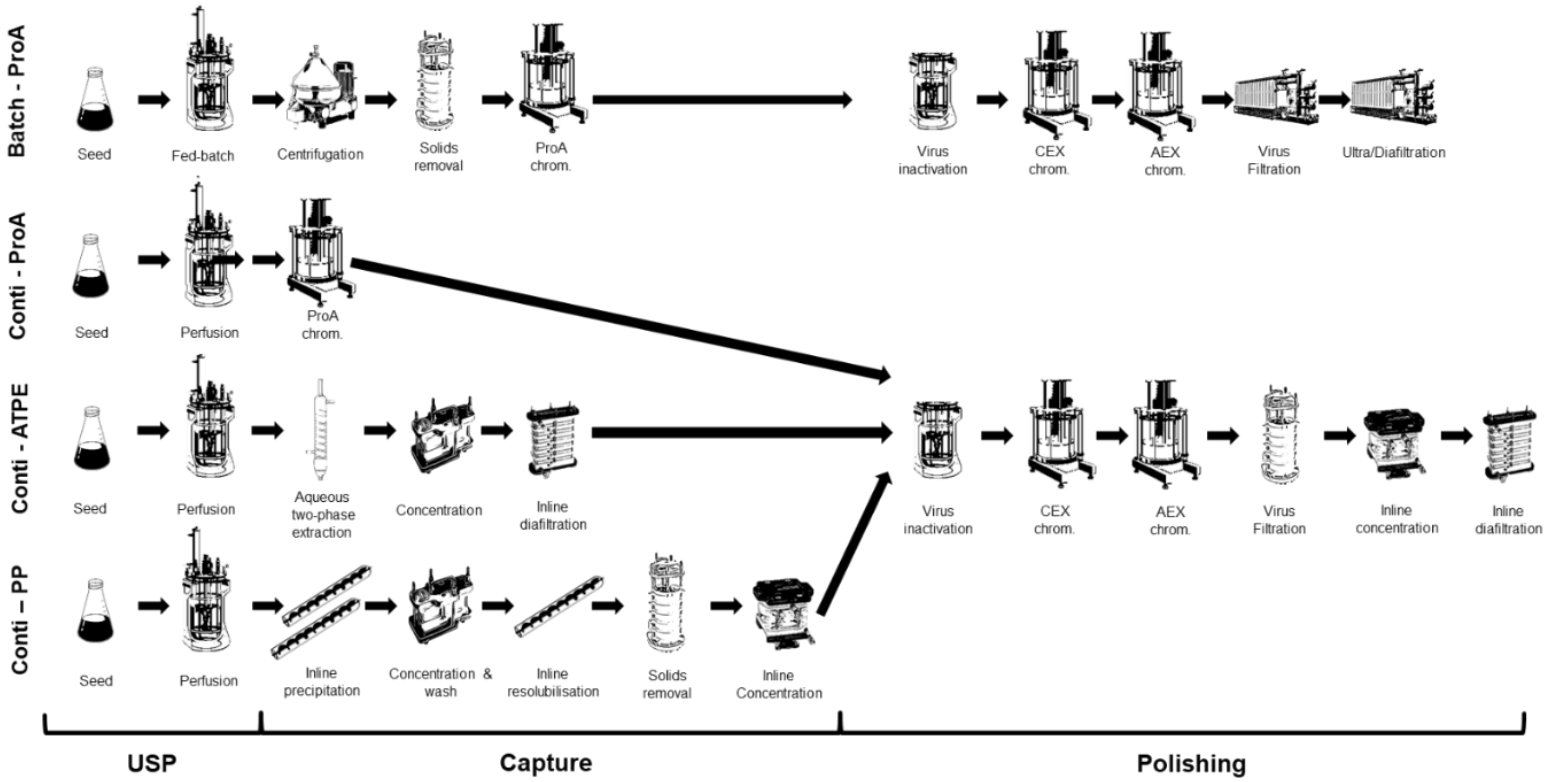


Figure 3.1 - Process flowsheets studied in batch and continuous production of monoclonal antibodies. ProA: protein A; CEX: cation exchange; AEX: anion exchange; TFF: tangential flow filtration; SP-TFF: Single-pass tangential flow filtration. Batch-ProA: batch mAb production with protein A as capture step; Conti-ProA: continuous mAb production with protein A chromatography as capture step; Conti-ATPE: continuous mAb production with aqueous two-phase extraction as capture step; Conti-PP: continuous mAb production with product precipitation as capture step. Batch success rate is considered 96% across flowsheets.

Table 3.1 - Process-specific input assumptions in the COG model for Batch-ProA, Conti-ProA, Conti-ATPE and Conti-PP flowsheets.

Unit operation	Parameter	Batch	Continuous
Cell culture	Culture Duration (days)	14	28
	Perfusion rate (vv/day)	-	1.5
	Volumetric productivity ($g/L_{vv}/d$)	0.5	3
	Max bioreactor volume (L)	20 000	2 000
	Collected Titre ($g/L_{harvest}$)	5	2
	Batches per year, $N_{batches}$	20	10
Protein A chrom.	Bed height, BH (cm)	20	10
	Loading capacity, DBC (g/L_{resin})	40	65
	Linear velocity (cm/h)	350	180
	Number of columns, N_{col}	1	3
	Resin reuse limit, N_{reuse} (cycles)	200	200
ATPE	HCCF (%)	-	18
	PEG (%)	-	9.6
	Phosphate (%)	-	13
	NaCl (%)	-	10
	$Ratio_{top/bottom}$	-	0.4
Precipitation	HCCF (%)	-	50
	PEG (%)	-	7
	$ZnCl_2$ (%)	-	10
VI	Concentration into VI (g/L)	~18	31.5
Final UFDF	Final target concentration (g/L)	30	30

Notes: Collected titre is measured in grams of product per litre of harvested cell culture fluid. Volumetric productivity is measured in grams of product produced per litre of the bioreactor working volume per day. The perfusion productivity, loading capacity and bed heights are taken from Mahal (2021). The fed-batch productivity (0.5 g/L/day) is derived based on the collected titre of 5g/L divided by 10 days of fed-batch expansion (after a 4-days ramp-up phase). The perfusion rate is measured as the equivalent number of bioreactor vessel working volumes (vv) exchanged per day. The higher mAb concentration after ProA elution into the virus inactivation step in continuous mode results from the higher loading capacity (65 vs 40 g/L resin) for the same eluted column volumes (CVs batch & continuous: 4 equilibration, 5 elution, 2 wash, 3 strip, 3 regeneration)

A total 14-day fed-batch process with a 5 g/L titre was compared with a total 28-day perfusion in the continuous mAb production strategies. For continuous perfusion, the product collection starts after the initial growth and ramp-up phase of 8 days (versus 4 days in fed-batch) and a 6-fold increase in volumetric productivity (3 g/L/d) was assumed over batch. For continuous multicolumn chromatography, higher resin loading capacities (ProA DBC=65g/L resin, AEX DBC=100g/L resin, CEX DBC=100g/L resin) were assumed given the better resin capacity utilisation compared to batch (Pollock et al., 2013; Jagschies, 2018). The binding capacities and prices for protein A resin in the different modes were selected to reflect the latest industry benchmarks. A 3 column system is assumed in continuous chromatography. While column #1 is in loading mode, column #2 collects the unbound material (therefore the higher capacities assumed in continuous). Column #3 is used to collect the unbound materials from column #2 once this starts to be loaded when column #1 reaches capacity and enters the elution phase,

For continuous ATPE and continuous precipitation, the percentage of HCCF (percentage of the final volume in the ATPE/PP system corresponding to the perfusion broth volume after adding the other components, such as PEG or salt) was assumed as 18 (Rosa et al., 2012) and 50% (Li et al., 2019), respectively. The yield of continuous ProA chromatography in Conti-ProA was set as 95%, while the base case recovery of the ATPE step was 85% (Rosa et al., 2012) and the wash yield in Conti-PP was 82% (Li et al., 2019). The resulting overall DSP yields were of 70, 60 and 55% for Conti-ProA, Conti-ATPE and Conti-PP, respectively. The product concentration prior to the viral inactivation step in Conti-ATPE and Conti-PP was set as the same found after protein A chromatography in Conti-ProA (32 g/L), so the size and time of the polishing stage would be kept constant across continuous flowsheets.

Some authors have shown comparable purity (Azevedo, Rosa and Ferreira, 2008; Rosa et al., 2012) when using alternatives to ProA chromatography in mAb capture, others state further work is required to improve impurity removal (Li et al., 2019). In this work, the economic potential of the flowsheets was explored on the basis that all flowsheets are able to meet the target purity specifications.

The batch and continuous labour requirements are as described by Mahal et al. (2021) and 3 shifts per day are assumed with 6 operators per USP and per DSP shifts in Batch-ProA and 3 operators per USP and per DSP shifts in Conti-ProA, Conti-ATPE and Conti-PP. In this study, the definition of “batch” in the continuous flowsheets is taken as the quantity of product delivered per cell culture run (10 batches per year). Similarly to what is described in Mahal, Branton and Farid (2021), as a quality control batch release test in continuous is performed every four days on the material collected in that period of time, there are 5 “QC batches” per perfusion culture (perfusion expansion phase of 20 days), which is taken into account when calculating the QC costs (35k$ per batch release test).

In the present model, all process buffers were purchased for a fixed cost (no buffer preparation in-house). In single-use facilities, these buffers are stored in single-use bags (maximum capacity of 5000 L) and bag containers and trolleys are required to hold them in place where needed throughout the process train. These containers were considered in the indirect costs as part of the equipment purchase cost used in the calculation of the fixed capital investment.

3.2.1.1 Uncertainty assumptions

One-way sensitivity analyses were used to identify the key COG drivers. These were then used in the Monte Carlo simulations. The Monte Carlo simulation algorithm was coded in Python to enable distributions (e.g., triangular) to be applied to the designated input parameters and used a random number generator to create the set of iterations. The algorithm computed the likelihood of the COG output falling below different thresholds. A two-tailed t-test was performed to evaluate whether there was a significant difference between the COG/g distributions of the flowsheets, as indicated by the p-values and a chosen significance level of 0.05. The algorithm was used to perform 100 iterations per run which was found to be sufficient to reach convergence. The number of iterations needed to reach convergence was determined by calculating the mean and standard deviation after each run (from n=2) and monitoring when these values were within a tolerance of 5% from the global

mean and standard deviation. The global mean and standard deviation corresponded to the values calculated after 1000 runs.

Typical cell culture titre fluctuations are of ±20% (Pollock, Ho and Farid, 2013). This variation was also applied to the dynamic binding capacity (DBC) of protein A, as a way of simulating the influence that different required quantities of expensive resin could have on the cost of goods. The specific column-free alternatives' parameters and ranges were discussed with experts in these technologies. Variations at the cell culture tire or volumetric productivity were translated into smaller/bigger/more/fewer bioreactors required and smaller/larger media consumption. The same effect was seen when considering uncertainty in the process step yields, as the USP was redesigned to compensate for the product gain/loss during the downstream processing. The variation of the HCCF percentage in ATPE and PP impacted the dilution of the broth coming from perfusion, thus, the burden on the concentration steps required before the virus inactivation.

Table 3.2 - Triangular distributions used in Monte Carlo simulations for the uncertainty analysis. HCCF % and respective distributions are different for ATPE and PP based on different research papers in which the model was based and discussion with the respective authors.

Parameter	**Distribution**	**Flowsheet**
Fermentation titre (g/L)	Tr (3.75,5,6.25)	Batch-ProA
Perf. Volumetric productivity (g/L/day)	Tr (2.25, 3, 3.75)	Conti-ProA/ATPE/PP
ProA Dynamic binding capacity (g/L resin)	Tr (50, 65, 80)	Conti-ProA
ATPE yield	Tr (0.80, 0.85, 0.90)	Conti-ATPE
HCCF% ATPE	Tr (0.14, 0.18, 0.25)	Conti-ATPE
HCCF% PP	Tr (0.40, 0.50, 0.60)	Conti-PP
Wash yield	Tr (0.75,0.82, 0.90)	Conti-PP

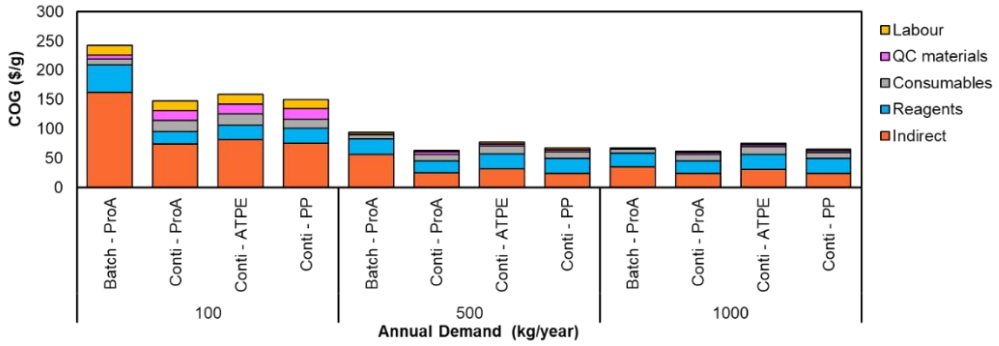
3.3 Results and discussion

The attractiveness of batch and continuous manufacturing strategies with different capture technologies was assessed using a decisional tool that captured the nuances of different modes of operation and different technology choices. The cost analysis was extended by evaluating each process's environmental burden and using stochastic uncertainty analysis to assess the robustness of the different scenarios under inherent process variability. An MCDM analysis was used to weigh up the financial, environmental and operational attributes of each flowsheet. A final target analysis highlighted the process changes needed for alternative production strategies to become cost-competitive.

3.3.1 Deterministic Cost Analysis

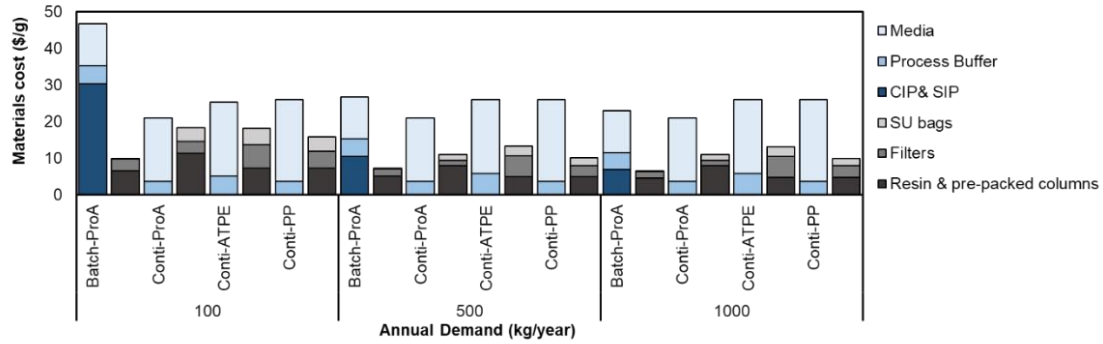
The COG/g outputs from the deterministic analysis conducted with the decisional tool are shown in Error! Reference source not found.**a** on a cost category basis for the batch and continuous mAb flowsheets. This figure shows that the continuous production flowsheets, whether ProA-based or column-free (Conti-ProA, Conti-ATPE and Conti-PP) could offer COG savings of ~20-40% compared to the standard batch flowsheet (Batch-ProA) at lower and medium scales (100 and 500 kg/year). In contrast, at higher scales (1000 kg/year) only Conti-ProA and Conti-PP presented a similar or slightly lower COG than Batch-ProA.

(a)



% difference in COG vs. Batch-ProA	-	-39%	-34%	-38%	-	-33%	-18%	-29%	-	-8%	+12%	-3%
% difference in COG vs. Conti-ProA	-	-	+8%	+2%	-	-	+22%	+6%	-	-	+22%	+6%
DSP yield	0.63	0.70	0.68	0.55	0.63	0.70	0.68	0.55	0.63	0.70	0.68	0.55
Reactor size (kL)	2	0.3	0.3	0.4	10	1.5	1.5	2	20	1.5	1.5	2
ProA chrom. scale (# columns, diameter in cm; CV in L)	1;45;32	3;13;1.2	-	-	1;80;100	3;25;5	-	-	1;110;190	3;25;5	-	-
Filtration (SPTFF) area (m^2)	-	-	3.5	1.5	-	-	37.5	7.5			37.5	7.5
Batches/campaign	20	10	10	10	20	10	10	10	20	20	20	20
Parallel trains	1	1	1	1	1	1	1	1	1	2	2	2
FCI ($M)	56	25	28	25	97	42	54	41	124	85	108	83
% indirect in total COG	67%	50%	51%	50%	59%	39%	40%	36%	53%	40%	41%	37%
% reagents in total COG	19%	14%	16%	17%	28%	33%	34%	39%	34%	34%	35%	40%
% consumables in total COG	4%	12%	12%	11%	8%	17%	17%	15%	9%	18%	18%	15%

(b)



% difference in reagents costs	vs. Batch-ProA	-	-55%	-44%	-44%		-22%	-3%	-3%		-8%	+13%	+13%
	vs. Conti-ProA	-	-	+24%	+24%		-	+24%	+24%		-	+24%	+24%
% difference in consumables costs	vs. Batch-ProA	-	+84%	+83%	+59%	-	+56%	+88%	+43%	-	+73%	+109%	+58%
	vs. Conti-ProA	-	-	+1%	-14%	-	-	+20%	-9%	-	-	+20%	-9%
% difference in equipment costs	vs. Batch-ProA	-	-55%	-41%	-54%	-	-55%	-15%	-53%	-	-30%	+33%	-27%
	vs. Conti-ProA	-	-	+33%	+4%	-	-	+90%	+4%	-	-	+90%	+4%

Notes: At 1000 kg/year, two parallel trains are implemented in continuous mode; therefore, the number of batches, stainless equipment and SU materials are doubled. The fed-batch flowsheet is integrated in a stainless-steel based facility, while the continuous flowsheets are single-use based. The titre for fed-batch culture is 5g/L and the perfusion volumetric productivities assumed in all continuous strategies was 3g/L/day. SU bags include both bioreactor bags and buffer hold bags. The embedded table in (a) indicates the key parameters for each batch and continuous facility and the percentage of indirect, reagents and consumables in each flowsheet's COG/g. Different filtration areas in ATPE and PP's SPTFF steps are a result of different volumes (higher dilution in ATPE) to be concentrated. The embedded table in (b) presents the percentage difference in equipment, reagents and consumables costs between flowsheets.

Figure 3.2 - Breakdown of (a) COG/g on a cost category basis and (b) materials (reagents and consumables) cost for four mAb production flowsheets at 100, 500 and 1000 kg/year commercial scales. Mahal et al. (2021) presents results for the Batch-ProA and Conti-ProA flowsheets for demands up 3000 kg/year.

The cost savings with continuous flowsheets relative to batch at low and medium scales were driven by savings in indirect and reagent costs. The decrease in indirect costs can be attributed to the smaller equipment needed in continuous mode given the higher cell culture productivities in perfusion; e.g. at 500kg/year the indirect costs change from 56$/g for Batch-ProA to 25$/g for Conti-ProA, 31$/g for Conti-ATPE and 24$/g for Conti-PP. The savings in equipment costs switching from Batch-ProA to the continuous flowsheets could go up to 50% for 7-fold productivity differences. Also, as shown in Error! Reference source not found.**b**, the savings associated with the absence of CIP cleaning procedures in SU facilities outweighed the 2 to 3-fold higher media consumption found for perfusion bioreactors and led to a 10 to 60% cost reduction in reagents across scales and flowsheets (e.g. 500kg/year reagents costs: 27$/g Batch-ProA, 21$/g Conti-ProA, 25$/g Conti-ATPE, 26$/g Conti-PP). Media consumption costs are higher in continuous due to higher perfusion media prices and volumes. Hybrid SU facilities operated in batch mode have been reported by Mahal (2021) and showed benefits over stainless steel based batch facilities at lower (100 kg/year) and medium (500 kg/year), but always higher COG compared to SU facilities operated in continuous mode for mAb manufacture. The savings in indirect and reagent costs were more significant than the increase in consumables costs (up to 2-fold) when using single-use continuous flowsheets (e.g. 500kg/year consumables costs: 7$/g Batch-ProA; 11$/g Conti-ProA; 13$/g Conti-ATPE, 10$/g Conti-PP).

Turning to the comparison at the higher 1000 kg/year demand, the lower COG savings with the continuous flowsheets were due to the need for multiple (two) parallel production trains. As the capacity of the SU bioreactor bags is limited (maximum capacity assumed was 2000L), when more than one bioreactor was required, an additional dedicated DSP train was simulated in parallel. At 1000 kg/year, Conti-ProA and Conti-PP presented COG savings of 8% and 3%, respectively, compared to Batch-ProA, which were not so significant given the typical accuracy of cost estimates. Due to the comparable indirect costs and higher consumables costs of Conti-ATPE compared to Batch-ProA at high scale, the ATPE-based flowsheet showed a COG/g increase of more than 10%

compared to the batch case; this was the only scenario where a continuous flowsheet was found to perform worse than the batch flowsheet.

The cost comparison among continuous flowsheets in Error! Reference source not found.**a** also showed that Conti-ProA was the strategy offering the lowest COG/g across scales, followed closely by Conti-PP (2-6% higher COG). Conti-ATPE presented the highest COG/g (8-22% higher COG than Conti-ProA) amongst all continuous flowsheets. For Conti ATPE, all cost categories were higher than Conti-ProA. This is mainly attributed to the HCCF dilution that drives up equipment costs (bag containers and filtration skids), consumables (SU bags) and reagents (ATPE-specific buffers and diafiltration buffers). For Conti PP, the overall cost was similar or slightly higher than Conti-ProA. While the reagents costs were 24% higher, driven by the larger media volumes (30% higher media costs than Conti-ProA), the consumables costs were lower (9-14%), mainly due to the absence of ProA resin, and the total equipment purchase cost was similar.

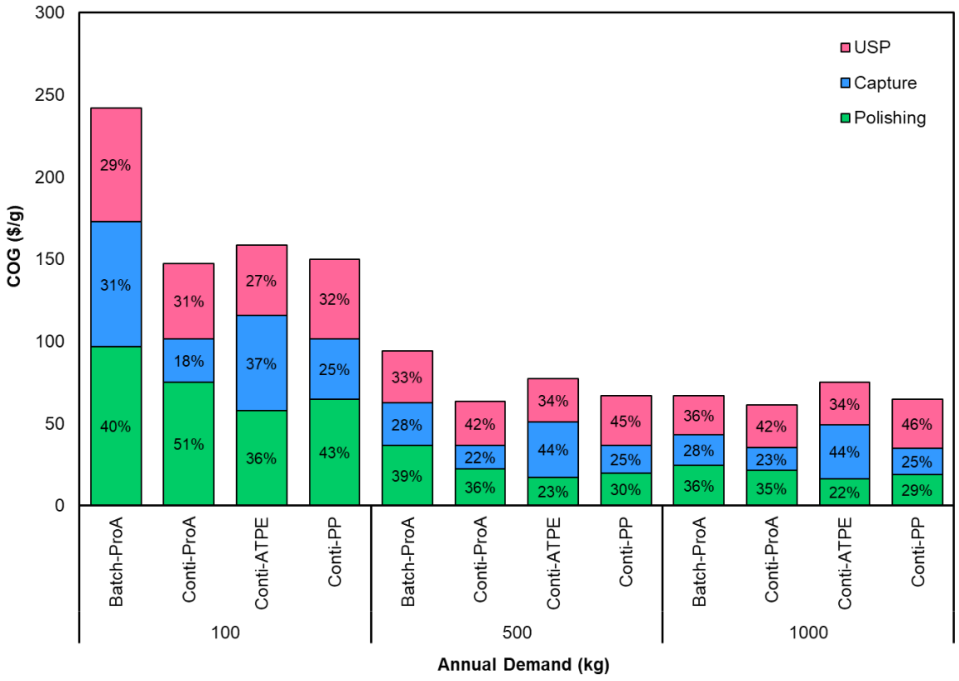


Figure 3.3 - Breakdown of COG/g per processing stage of four mAb production flowsheets. The COG breakdown on a process stage basis is showed for 100, 500 and 1000kg/year commercial scales, while the contribution of each process stage in each cost category is shown for the 500kg/year scale. USP and

polishing steps are fixed among continuous flowsheets, while the capture stage comprises different unit operations (as presented in **Figure 3.1**).

Error! Reference source not found. depicts the COG breakdown by major stage (USP/capture/polishing). This highlights that the steps involved in the capture stage represent a significant proportion of the COG with a base value of ~30% for the conventional batch flowsheet. Moving from the conventional batch flowsheet (Batch-ProA) to continuous flowsheets with chromatography (Conti-ProA) or precipitation (Conti-PP) results in a reduction in the contribution of the capture stage to the overall COG from ~30% to <25%. In contrast, the continuous ATPE flowsheet (Conti-ATPE) results in a higher contribution of the capture stage to the overall COG than any of the other strategies (37-44%).

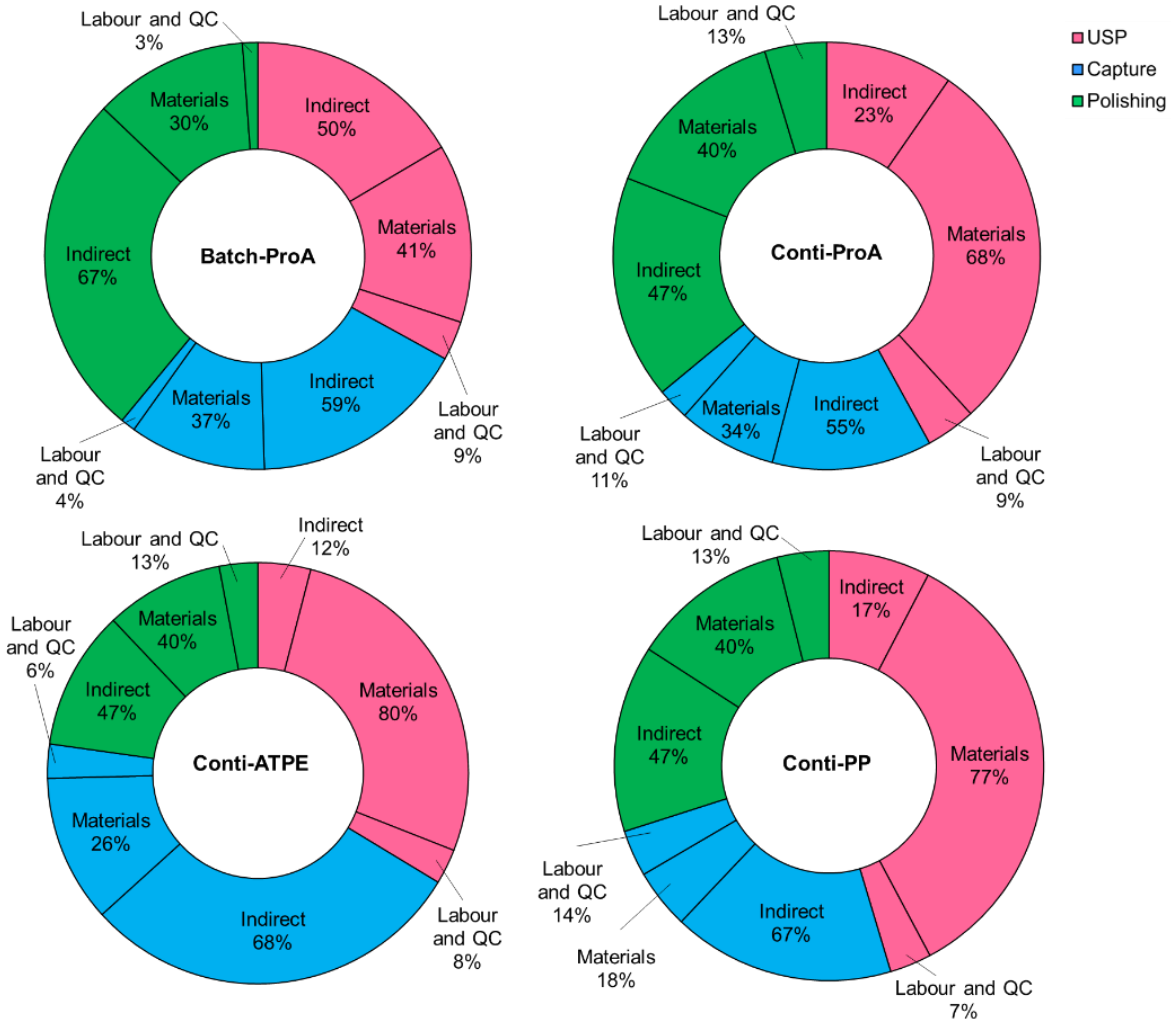


Figure 3.4 - Breakdown of impact of each cost category in the different manufacturing stages of four mAb production flowsheets for the 500kg/year

scale. USP and polishing steps are fixed among continuous flowsheets, while the capture stage comprises different unit operations (as presented in **Figure 3.1**).

Error! Reference source not found. provides benchmark cost category distributions by stage at 500 kg/year, focusing on the stages most affected by the change in strategy – USP and capture. For the capture, both batch and continuous processes are dominated by the indirect costs that consume over 50% of the capture COG (COG capture distribution = 19-37% Materials, 4-14% Labour and QC, 55-68% Indirect); hence for capture stages, changes in capital equipment will have a larger impact than changes in materials, such as the resin. In contrast, for the USP stage, the move from batch to continuous flowsheets results in a shift in the USP COG distribution from being dominated by USP indirect costs (50%) in batch processes (COG USP batch distribution=41% Materials: 9% Labour: 50% Indirect), to USP materials (~70-80%, predominantly culture media reagents) in continuous processes (COG USP continuous distribution = 68-77% Materials: 7-9% Labour and QC, 12-23% Indirect). As the polishing steps are kept constant across the continuous strategies, the contribution of indirect, materials and labour and QC costs are identical.

Table 3.3 shows the items that contributed the most for each cost group in the capture stage only at a demand of 500 kg/year. In the equipment costs, which are directly related to the indirect costs, the centrifuge and chromatography skids in Batch-ProA and Conti-ProA, respectively, are the most expensive items in the capture step. Regarding the column-free capture alternatives, one can confirm the significant contribution of bag containers for the total equipment cost in Conti-ATPE and Conti-PP capture, due to the large volumes associated with the HCCF dilution and multiple filtration steps in these flowsheets. In the reagents and consumables front, buffers and resin weight the most in Batch-ProA and Conti-ProA materials cost and it is also possible to observe the significant portion of CIP buffer costs in Batch-ProA capture costs (59%). For Conti-ATPE and Conti-PP, the membranes used in the filtration steps and the specific reagents (e.g., PEG, HEPES) sum the major materials in costs.

	Batch-ProA	Conti-ProA	Conti-ATPE	Conti-PP
EQUIPMENT				
Centrifuges	26%	N/A	N/A	N/A
Bag containers	N/A	14%	94%	86%
Bag trolleys	N/A	<1%	<1%	<1%
Hold-tanks	53%	N/A	N/A	N/A
Filtration skids/pumps	1%	N/A	4%	13%
Chromatography skids	16%	86%	N/A	N/A
Chrom. columns (glass)	4%	N/A	N/A	N/A
Extractor	N/A	N/A	1%	N/A
REAGENTS				
CIP buffer	59%	N/A	N/A	N/A
PW & WFI	7%	0%	20%	26%
Buffers	35%	100%	28%	10%
ATPE/PP specific reagents	N/A	N/A	53%	64%
CONSUMABLES				
Guard filters	8%	N/A	N/A	N/A
Hold bags	N/A	8%	27%	40%
Filters	7%	N/A	73%	60%
Packed columns and resin	85%	92%	N/A	N/A

Table 3.3 - Contribution of each equipment, reagents and consumables item for the total cost of the capture step only. N/A: not applicable (USP and polishing steps not included).

3.3.2 Environmental Analysis

The potential environmental benefits moving from batch to continuous and from column-based to column-free mAb capture were evaluated by analysing the environmental burden associated with each mAb flowsheet. The process mass intensities (PMIs) are shown in **Figure 3.5** and are split into water and consumables PMI for the different production strategies.

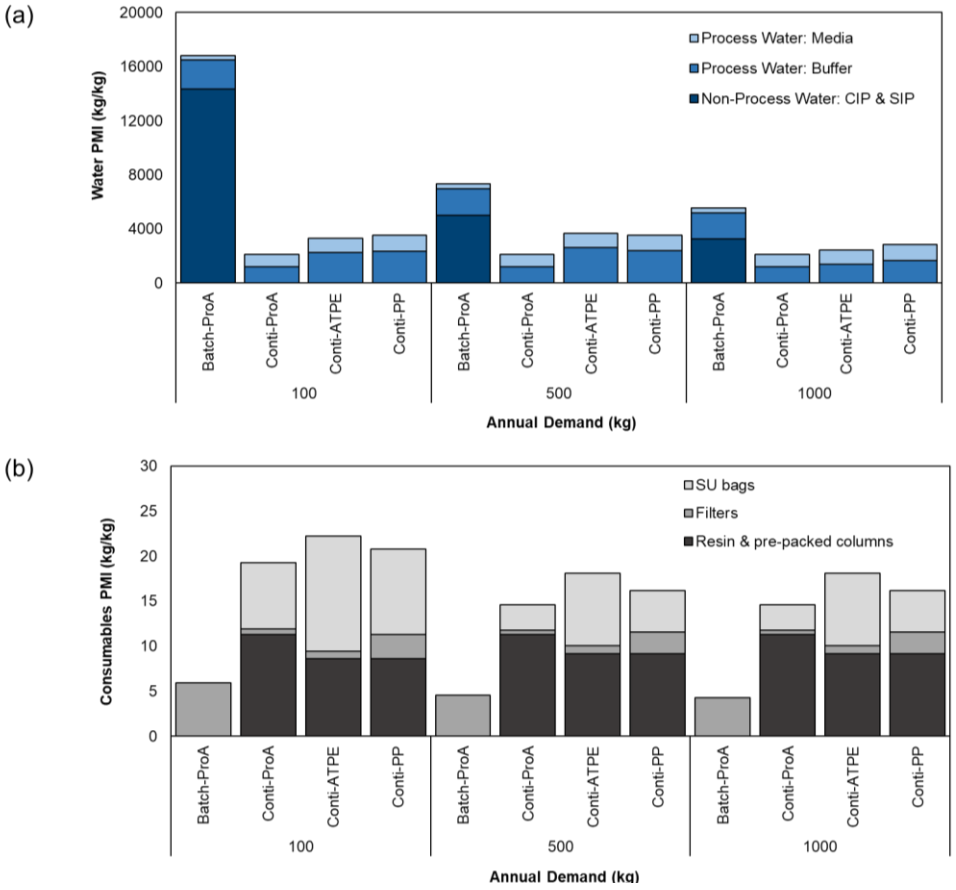


Figure 3.5 - a) Water and b) consumables process mass intensity (PMI) breakdown for four mAb production flowsheets at 100, 500 and 1000 kg/year commercial scales. The water and consumables PMIs include the complete production train liquid and solid waste, respectively. The consumables PMI is based on the total weight of individual disposable material (SU bags, filters, resin and pre-packed columns). The weight of each material was found in literature or given by suppliers. SU bags include both bioreactor bags and buffer hold bags.

Continuous flowsheets offered a significant reduction in the overall PMI compared to the traditional batch process depending on scale, with Conti-ProA offering the most environmentally friendly strategy of all mAb production strategies. This was driven by the reduction in water PMI that outweighed any increases in consumables PMI since water PMI values were in the order of thousands of kg/kg while consumables PMI were significantly lower and in the order of tens of kg/kg.

Digging deeper into the analysis produces useful PMI benchmark values for the sector. Water PMIs for Batch-ProA were between 5000 and 17,000 kg/kg, while consumables PMIs ranged between 4 and 6 kg/kg, depending on the production scale. According to **Figure 3.5a**, the switch from batch to continuous flowsheets can lead to 2-8-fold lower water PMIs from high to low production scales (2200 kg/kg Conti-ProA, 3300 kg/kg Conti-ATPE, 3600 kg/kg Conti-PP across scales). As discussed in the cost analysis, the absence of CIP procedures in continuous single-use based facilities results in significant water savings compared to the batch stainless-steel based strategy. Although the continuous options have a lower water PMI than the batch flowsheet, the continuous column-free options fare worse than the continuous ProA option. The higher water PMIs of Conti-ATPE and Conti-PP compared to Conti-ProA can be attributed to the higher media consumption and diafiltration buffers.

In contrast to the water PMI trends, the consumables PMI in **Figure 3.5b** was 4 to 5-fold higher in continuous mode (e.g. 500 kg/year consumables PMI: 5 kg/kg Batch-ProA, 15kg/kg Conti-ProA, 18 kg/kg Conti-ATPE, 16 kg/kg Conti-PP). However, the order of magnitude is negligible compared to the lower liquid waste. On the consumables front, as expected from the cost analysis, Conti-ATPE resulted in a higher consumables PMI (SU bags and membranes). Regarding Conti-PP, contrary to the consumables cost savings compared to Conti-ProA, the consumables PMI in Conti-PP was in fact higher than the column-based option. This comes from the higher usage of filters and SU bags that outweigh the reduction in consumables weight (kg) from the absence of Pro-A pre-packed columns. Overall, Conti-ATPE presents a water PMI and a consumables PMI approximately 70% and 20% higher than Conti-ProA,

respectively, and Conti-PP presents a water PMI and consumables PMI approximately 60% and 10% higher than Conti-ProA, respectively.

The water and consumables PMI values are within the range of values reported in the literature for continuous mAb flowsheets (Ho et al., 2010; Pollock et al., 2017; Madabhushi et al., 2018; Cataldo et al., 2020) and suggest that continuous and single-use technologies can be key enablers for improving environmental impact in terms of overall PMI.

3.3.3 Sensitivity Analysis

In every large-scale bioprocess there are inherent uncertainties; thus, it is important to identify the key sources for technical deviations and account for them in the cost model to generate representative results. While the economic and environmental advantages of pursuing Conti-ProA strategy were highlighted during the deterministic cost comparison, a stochastic analysis enabled the evaluation of different scenarios under process variability. The first step was to conduct a sensitivity analysis by changing some critical process parameters in the column-based and column-free capture flowsheets to understand which factors had the largest influence on mAb production costs and to identify the major risks or benefits for production in terms of process changes. The selected ranges presented were discussed with academic and industrial partners, so the analysis could fairly represent the best and worst technical parameters found for each one of the technologies.

The results of the sensitivity analysis are illustrated in the tornado diagrams in **Figure 3.66**. The diagrams illustrate that the key COG driver is the titre (in Batch-ProA) and volumetric productivity (in Conti-ProA/ATPE/PP) in cell culture. Lower titres/productivities than expected resulted in higher USP costs as larger or more bioreactors were required to meet the demand. This had a knock-on impact on total reagent costs, dominated by CIP buffer costs in Batch-ProA (58%) and media costs in the continuous strategies (>75%). On the other hand, working with increased volumetric productivities and more concentrated HCCF would benefit specially the column-free alternatives, as lower perfusion volumes would require a smaller DSP.

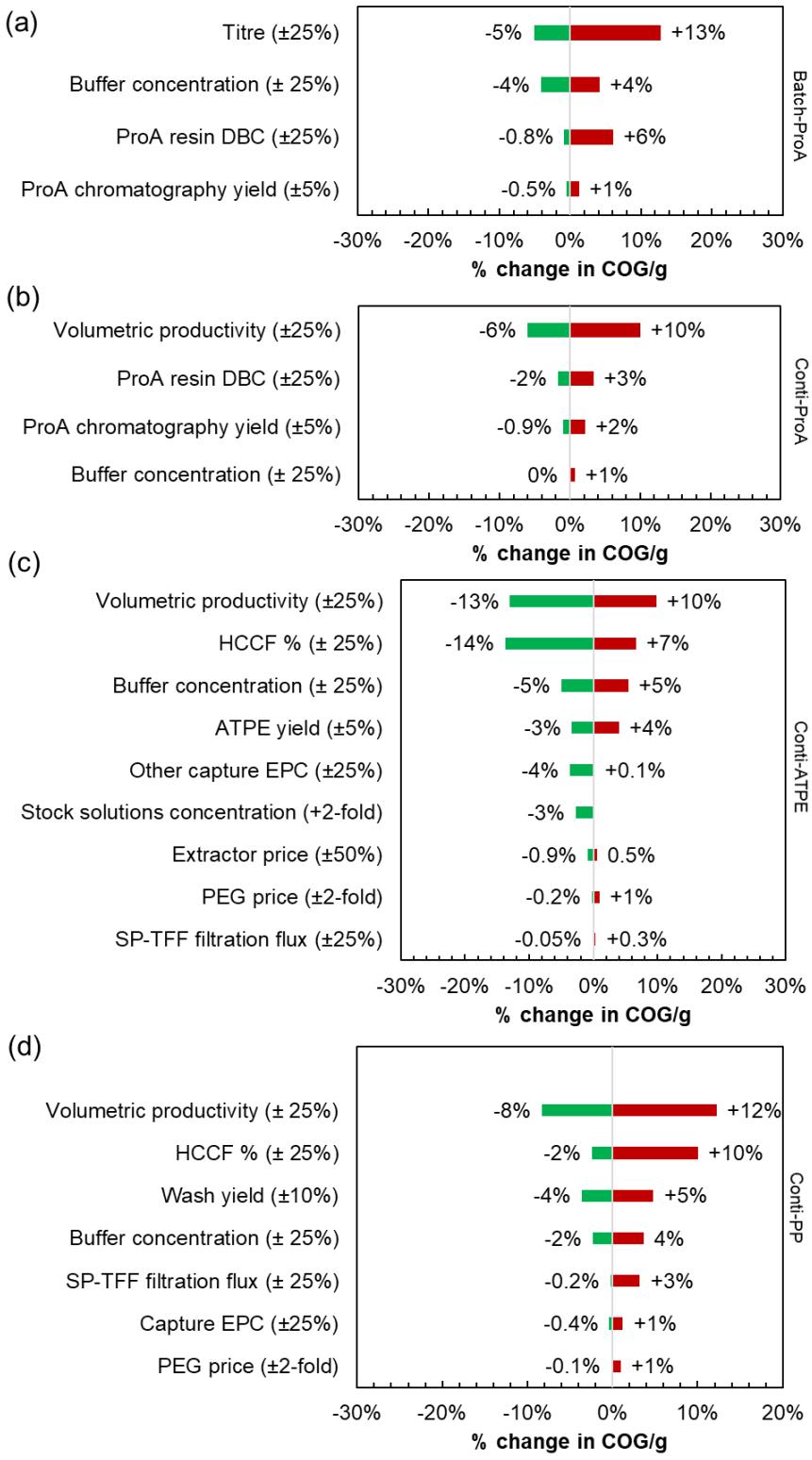


Figure 3.6 - Sensitivity analysis of COG/g showing the effect of process parameters variation on a) Batch-ProA, b) Conti-ProA, c) Conti-ATPE or d) Conti-PP mAb production flowsheets, at 500 kg/year scale. The percentage differences are relative to the COG/g in the base case.

Figure 3.6c and **Figure 3.6d** show that the HCCF percentage was the second parameter with the largest impact on the cost of goods in both column-free capture alternatives. The HCCF% in the ATPE or PP systems' composition determined the dilution of the broth and the volume handled in the following steps. Therefore, changes at this level had a large impact on the equipment investment (associated with the indirect costs), consumables and reagents costs. In Conti-ATPE, an increase from 18% to 25% of perfusion liquid in the ATPE system (HCCF%) could decrease the final COG/g by more than 10% at medium and large scales, demonstrating the benefits of a lower product dilution on the cost-effectiveness of liquid extraction for mAb capture.

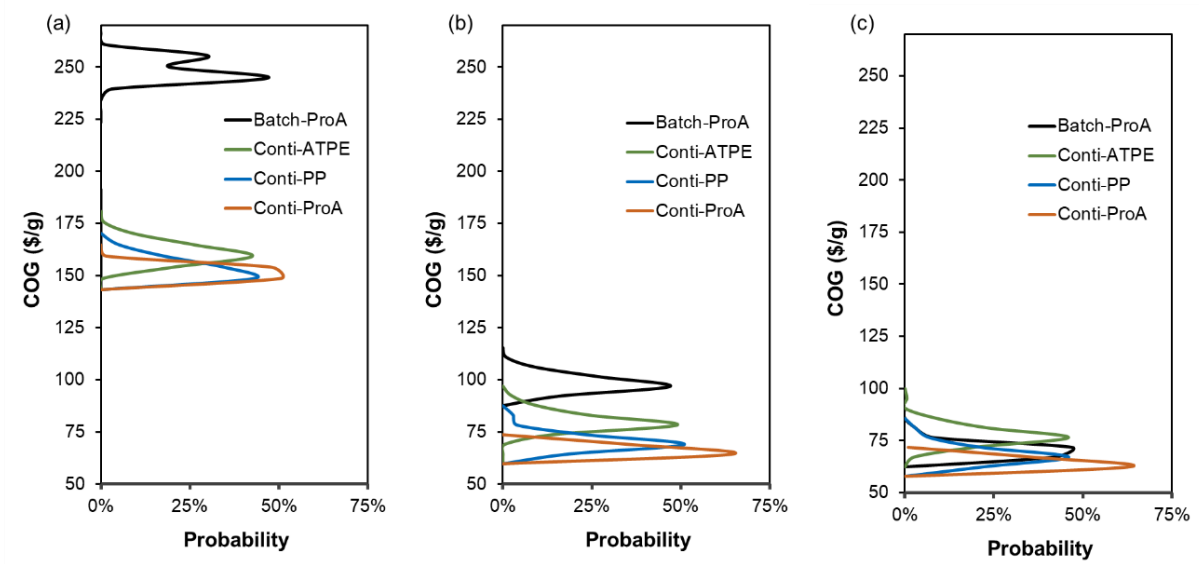
The diagrams illustrate that the key COG driver is the titre (in Batch-ProA) and volumetric productivity (in Conti-ProA/ATPE/PP) in cell culture. Lower titres/productivities than expected resulted in higher USP costs as larger or more bioreactors were required to meet the demand. This had a knock-on impact on total reagent costs, dominated by CIP buffer costs in Batch-ProA (58%) and media costs in the continuous strategies (>75%). On the other hand, working with increased volumetric productivities and more concentrated HCCF would benefit specially the column-free alternatives, as lower perfusion volumes would require a smaller DSP. **Figure 3.6c** and **Figure 3.6d** show that the HCCF percentage was the second parameter with the largest impact on the cost of goods in both column-free capture alternatives. The HCCF% in the ATPE or PP systems' composition determined the dilution of the broth and the volume handled in the following steps. Therefore, changes at this level had a large impact on the equipment investment (associated with the indirect costs), consumables and reagents costs. In Conti-ATPE, an increase from 18% to 25% of perfusion liquid in the ATPE system (HCCF%) could decrease the final COG/g by more than 10% at medium and large scales, demonstrating the benefits of a lower product dilution on the cost-effectiveness of liquid extraction for mAb capture.

The concentration of buffers was also a factor to be considered when looking at process changes that could reduce costs in column-free strategies. As the bag containers dominate the equipment costs for the capture sequence in Conti-ATPE and Conti-PP, using buffer concentrates and inline dilution would

bring savings in the final cost of goods. Other changes in parameters such as the reagents' price, filtration fluxes, or other equipment price (besides capture EPC) had a lower impact on the final COG/g; thus, they are not considered to portray significant risks for the process. The individual parameter changes that resulted in greater than a 5% change in COG were selected and integrated into the uncertainty analysis using Monte Carlo simulations, where the process mass output was fixed (100, 500 and 1000 kg/year) and the facility was resized for each iteration to reflect the consequences of different starting assumptions.

3.3.4 Uncertainty analysis with Monte Carlo simulations

Accounting for key uncertainties in the batch and continuous processes with a stochastic analysis enables the robustness of the options to be determined as well as the likelihood of meeting certain COG/g threshold values. The results of the stochastic Monte Carlo analysis are depicted in the COG frequency distributions in **Figure 3.7** with an embedded table of key statistics. The figure shows that Conti-ProA presented the most robust alternative across demands compared to the batch and continuous column-free options as indicated by its narrower distribution and lowest standard deviation and hence risk. It had also the lowest expected cost and the differences in COG distributions were found to be statistically significant, as indicated by all p-values being below 0.05 (embedded table). Of the column-free options, Conti-PP had the higher probability of matching Conti-ProA expected COG values, with a likelihood ranging from 10 to 30% across scales (embedded table). Bimodal distributions, with peaks occurring at different COG values for the same alternative and scale, were observed for Batch-ProA at 100 kg/year and Conti-PP at 500 kg/year, when there was a jump in bioreactor scale due to low titre or productivity, respectively. Apart from this scenario, uncertainties in titres, process yields, HCCF% or binding capacities did not represent major shifts in the most likely COG/g for each flowsheet (peaks from stochastic distributions are within 5% of costs attained in the deterministic analysis, as shown in the embedded table).



(d)

Demand (kg)	100				500				1000			
Flowsheet	Batch-ProA	Conti-ProA	Conti-ATPE	Conti-PP	Batch-ProA	Conti-ProA	Conti-ATPE	Conti-PP	Batch-ProA	Conti-ProA	Conti-ATPE	Conti-PP
Deterministic COG ($/g)	242	148	159	150	94	63	77	67	67	61	75	65
eCOG ($/g)	246	149	158	151	96	64	78	68	68	62	75	66
Standard deviation	5.2	2.5	4.7	4.3	4.2	1.8	4.2	3.9	3.2	1.7	4.3	4.2
p-value (Batch-ProA)	N.A	<0.001	<0.001	<0.001	N.A	<0.001	<0.001	<0.001	N.A	<0.001	<0.001	<0.001
p-value (Conti-ProA)	<0.001	N.A	<0.001	<0.001	<0.001	N.A	<0.001	<0.001	<0.001	N.A	<0.001	<0.001
$p(COG \leq eCOG_{ProA})$	0%	49%	1%	31%	0%	52%	0%	13%	1%	51%	0%	12%

Figure 3.7 - COG/g probability distribution plots under manufacturing uncertainty at a) 100 kg/year, b) 5000 kg/year and c) 1000 kg/year production scales. d) Statistical data on COG/g for the competing technologies under process variability across demands. The p-values were computed using a two-tailed homoscedastic t-test with an alpha value of 0.05; p-values below this value indicate a significant difference. p-value (Batch-ProA) and p-value (Conti-ProA) refer to the values when the COG distributions from each flowsheet were compared with that of Batch-ProA and Conti-ProA, respectively. eCOG = expected COG.

3.3.5 Multi-criteria decision making

MCDM analysis was used to reconcile economic, environmental and operational criteria and identify the most advantageous continuous strategy considering all perspectives. While the economic (COG/g and FCI) and environmental (water and consumables PMI) criteria were directly obtained as model outputs, the qualitative criteria (e.g., ease of scale-up, ease of validation) were derived from survey responses from academia and industry experts with experience in affinity chromatography, liquid-liquid extraction and precipitation used in mAb capture.

Table 3.4 summarises the key values used in the MCDM to compute the overall aggregate scores for each flowsheet (ProA chromatography, ATPE and PP), including the criteria weights, standardised ratings and weighted category scores. From the list of qualitative operational criteria, robustness was the most important metric, while ease of installation ranked last, based on the survey responses.

The radar chart in **Figure 3.8a** shows all standardised rating values for each criteria for each flowsheet and it was used to simplify the visualisation of the preferred flowsheet at each criteria. As expected, Conti-ProA scored the highest in all economic and environmental criteria. Moreover, it had the maximum score in two out of five operational criteria, including robustness, the most important metric. Conti-ATPE had very poor scores across all quantitative metrics due to its high COG/g, equipment cost, consumable usage and water consumption; however, its operational feasibility was reasonably high according to the qualitative scores given by experts. Conti-PP scored well in the economic criteria, whereas, operational-wise, it only showed high scores for the two least important criteria (ease of operation and installation).

To reconcile the competing outputs, the overall aggregate score was generated for each flowsheet across different combination ratios of the economic, environmental and operational categories (**Figure 3.8b** and **Figure 3.8c**). The resulting sensitivity spider plots illustrate how the ranking of the alternative continuous options changes depending on user priorities. The figures clearly illustrate that Conti-ProA was the preferred continuous strategy irrespective of

the relative importance of the economic, environmental or operational category scores. The ranking between the remaining column-free options of Conti-PP and Conti-ATPE depended on the weightings of the categories. When economic and environmental performance were prioritised, Conti-PP was preferred over Conti-ATPE for all combinations of these two categories (**Figure 3.8b**). However, when operational performance was brought into the picture as a key priority (**Figure 3.8c**) and weighed against economic savings, then a switch point occurred where the operational category was twice as important as the economic category ($R_{op} = 0.6, R_{eco} = 0.3$). When the operational benefits dominated in the final score above this threshold ($R_{op} > 0.6$) then Conti-ATPE become the preferred column-free option over Conti-PP.

3.3.6 Target Analysis

The earlier COG analysis showed that the continuous mAb facilities modelled with column-free capture technologies did not offer lower manufacturing costs compared to the column-based option (Conti-ProA). This section determines the cost reductions required for column-free alternatives to achieve a target COG saving threshold of at least 15% compared to the continuous flowsheet with ProA capture to justify the process change. The ATPE and PP process changes implemented were based on the parameters that had the highest impact on COG/g savings in the sensitivity analysis, namely the perfusion volumetric productivity with either the ATPE HCCF% or the PP wash yield.

Figure 3.9 displays the target analysis as a matrix of heatmaps across scales and buffer preparation methods to determine the windows of operation where parallel improvements in ATPE and PP flowsheets result in COG savings that meet the target threshold of 15% (highlighted by the region within the black solid lines).

Table 3.4 - Multi-criteria decision making summary of weights, ratings and overall aggregate weighted scores

Criteria category, *k*	Criteria, *i*	Rank	Weight, E_i	Normalised weight, w_i	Rating value, x_{ij}			Standardised rating, r_{ij}			Weighted category score, y_{jk}			Overall aggregate score, S_j (R_{eco}=0.8, R_{env}=0.1, R_{op}=0.1)		
					Conti ProA	Conti ATPE	Conti PP	Conti ProA	Conti ATPE	Conti PP	Conti ProA	Conti ATPE	Conti PP	Conti ProA	Conti ATPE	Conti PP
Economic (500Kg)	Cost of Goods ($/g)	1	1	0.5	63	77	67	100	0	75	100	0	85	97	5	75
	Fixed Capital Investment ($)	1	1	0.5	25 M	27 M	25 M	100	0	96						
Environmental (500 Kg)	Water PMI (kg/kg)	1	1	0.5	2 156	3 684	3 566	100	0	54	100	0	31			
	Consumables PMI (kg/kg)	1	1	0.5	15	18	16	100	0	8						
Operational	Robustness	1	5	0.33	4.4	4.0	2.5	84	75	38	72	54	36			
	Ease of scale-up	2	4	0.27	3.4	4.7	3.8	60	92	69						
	Ease of validation	3	3	0.20	3.9	2.7	2.0	72	42	25						
	Ease of operation	4	2	0.13	3.3	2.7	3.5	56	42	63						
	Ease of installation	5	1	0.07	2.9	4.0	3.8	47	75	69						

Note: Rank of 1 indicates most important. For the operational metrics, a rating value of 5/5 represents the best outcome.

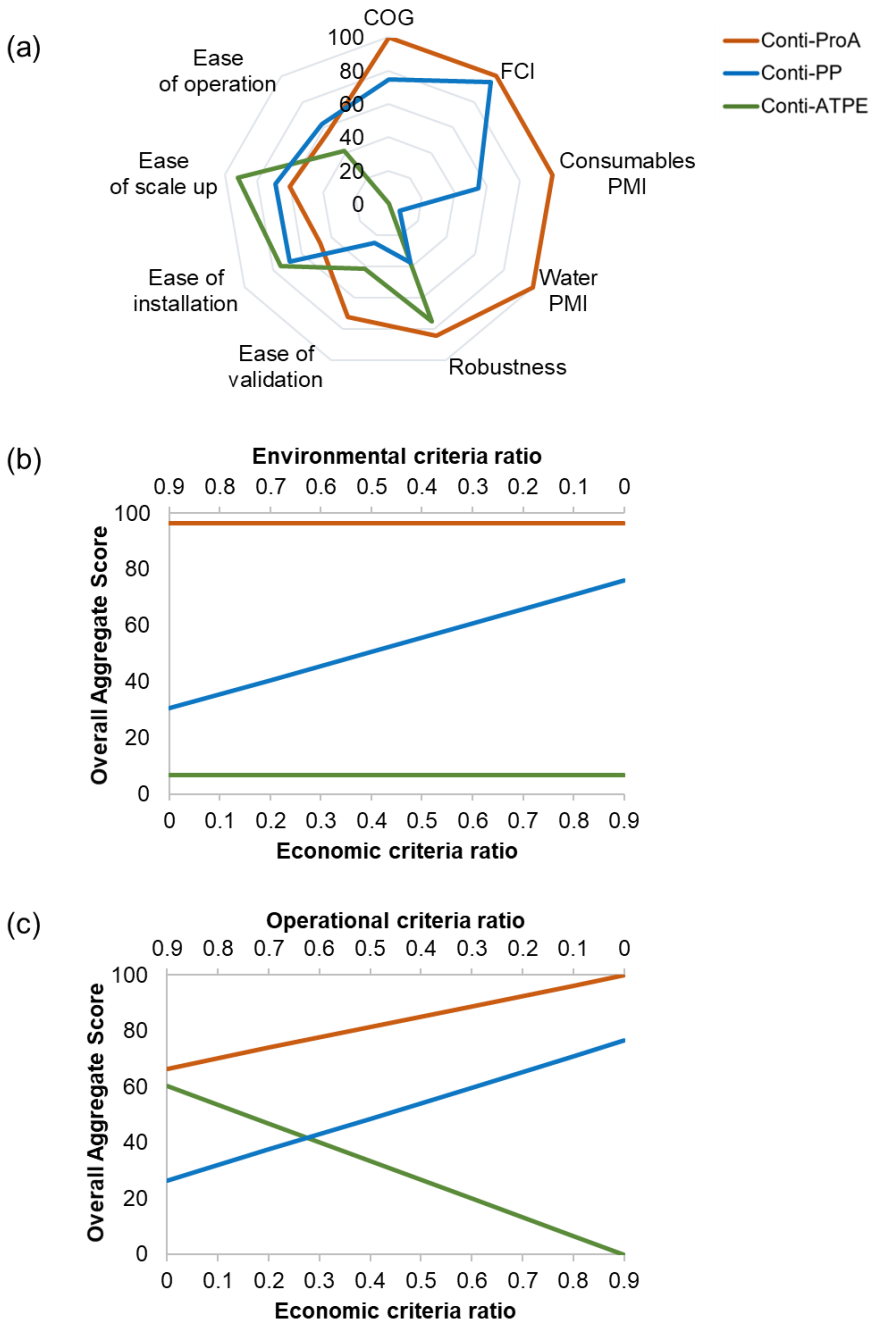


Figure 3.8 - Rating values of Conti-ProA, Conti-ATPE and Conti-PP flowsheets for each economic (Cost of Good – COG; Fixed Capital Investment – FCI), environmental (Consumables PMI; Water PMI) and operational (robustness, ease of operation, scale up, installation and validation) criteria. b and c) Effect of the economic, operational and environmental criteria combination ratios on the overall aggregate scores when the operational attribute ratio is constant at 10% (b) and when the environmental attribute ratio is constant at 10% (c). All graphs are generated for a mAb demand of 500 kg/year.

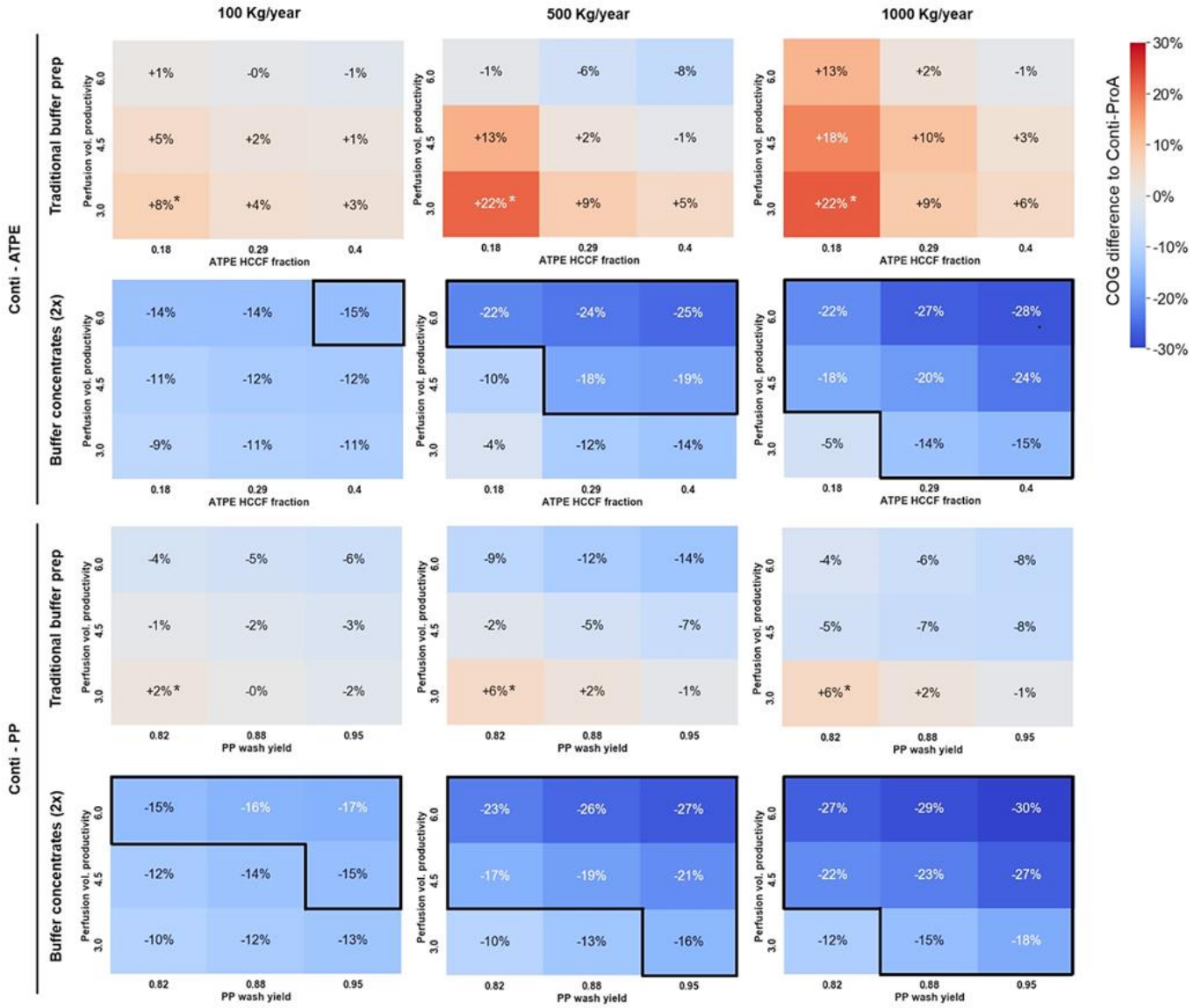


Figure 3.9 - Heat maps showing the COG difference for Conti-ATPE and Conti-PP relative to Conti-ProA as a function of the perfusion volumetric productivity versus either the HCCF% fraction for Conti-ATPE or the wash yield for Conti-PP. The target analysis is shown for scenarios using traditional buffer preparation as well as buffer concentrates. * indicates the base case scenario. The area within the solid black line indicates the conditions at which Conti-ATPE and Conti-PP present ≥15% COG/g savings compared to Conti-ProA.

With traditional buffer preparation, the target COG saving was not achieved regardless of the combination of parameters in the column-free flowsheets. Also, in Conti-ATPE, possible increases in volumetric productivity or HCCF fraction rarely led to COG values matching Conti-ProA COG. On the other hand, in Conti-PP, as the base-case COG is already very similar to Conti-ProA, changes in these process parameters led to scenarios offering modest savings over ProA chromatography.

The implementation of inline dilution of buffers (2-fold buffer concentrates) across all options conferred a strong advantage particularly for both Conti-ATPE and Conti-PP. The target COG saving could be reached for a broad combination of parameters in Conti-ATPE and Conti-PP. The window of feasible combinations meeting the target increased as scales increased due to the larger contribution of consumables and reagents costs to the total COG at higher scales and the ability of the process changes to minimise material consumption.

The attractiveness of Conti-ATPE and Conti-PP will depend on the improvement of multiple process parameters to levels that may be beyond the current best cases found in literature. The usage of buffer concentrates is becoming more commonplace and improved perfusion volumetric productivities may be envisioned for the near future.

Also, the implementation of a simple pre-concentration step before capture has been already discussed with partners and it would resemble the benefits of having higher volumetric productivities, as working with a more concentrated HCCF would lead to smaller volumes during DSP. However, increasing the HCCF percentage in the ATPE/PP systems without compromising capture performance and achieving higher step yields would entail further studies on the technical optimisation of aqueous two-phase extraction and precipitation as capture technologies applied to monoclonal antibodies.

3.4 Conclusion

This chapter presented the extent of capabilities configured in a process economics model that enabled the comparison of different mAb production flowsheets from economic, environmental and robustness perspectives. The simulation tool built in Python was used to design batch and continuous facilities and provided an in-depth evaluation of the trade-offs associated to protein A chromatography, aqueous two-phase extraction and product precipitation as mAb capture steps across production scales. The cost drivers for each scenario were highlighted and determined that the implementation of continuous manufacturing was preferable over batch, especially at lower scales, and that the broth dilution in ATPE and higher media consumption in PP could favour the selection of ProA as capture step in continuous mAb processing. Although there was an increase in consumables usage in continuous mode, the environmental analysis showed that the water savings found over batch would decrease the overall environmental burden associated with continuous mAb production. The multi-criteria decision-making analysis presented higher aggregate scores for continuous mAb processing with column-based capture across scenarios with different weightings for economic, operational and environmental performance. The target analysis showed that ATPE and PP could provide lower cost of goods than ProA if buffer concentrates are implemented and if the cell culture volumetric productivity, the HCCF% in the ATPE system and precipitates wash yields were maximised altogether. The added value of such a simulation framework was revealed through the assessment of different technologies, flowsheets and scenarios, as these are critical during process development and decision-making on future facility designs in the biopharmaceutical sector.

Chapter 4: Carbon footprint of different batch and end-to-end continuous antibody manufacture flowsheets

4.1 Introduction

As sustainability becomes a key focus for biopharmaceutical industry, measuring and reporting environmental metrics demonstrates not only an important commitment to the environment, but it can also help companies identify opportunities for improvement and make informed decisions about product design and manufacturing processes.

In this chapter, a cradle-to-gate life cycle analysis is carried out to determine the product carbon footprint resulting from different mAb manufacture flowsheets. The decision-support tool presented in the previous chapter was used to generate the mass balance and facility layout to feed into the environmental analysis. The PMI metrics from **Chapter 3**, which captured only waste generated, are compared with the sustainability metric related to product carbon footprint, which captures the impact of energy consumption, raw material extraction and waste treatment.

The key drivers of carbon footprint are identified and are used to provide an insightful overview of the areas in which industry should focus on when tackling process changes towards a more environmental-friendly production. Optimisation routes are also simulated to understand the potential decrease of carbon footprint in different mAb manufacturing flowsheets.

Section 4.2 presents the key assumptions and methodologies used during the life cycle assessment, including important approximations used in the database. **Section 4.3** starts by delving into the breakdown of the carbon footprint contributors and shares the environmental impact of each flowsheet, before and after process optimisation. **Section 4.4** reviews the main environmental outcomes shared during the chapter.

4.2 Methods

The case-study explored the life cycle assessment of three of the mAb flowsheets described in **Chapter 3**: Batch-ProA, Conti-ProA and Conti-PP. As Conti-ProA and Conti-PP were the end-to-end continuous flowsheets which presented the lowest COG/g and PMI, these were the focus of this in-depth environmental analysis.

The main attributes of the LCA are shown in **Table 4.1**. The assessment was performed according to the standards described in ISO 14040:2006 and 14044: 2006 (ISO, 2006a, 2006b).

Table 4.1 - Life Cycle Assessment main attributes

Software	OpenLCA
Database	EcoInvent 3.7
Impact Assessment Method	ReCIPe 2016 midpoint (H)
System Boundary	Cradle-to-gate
Scopes	1, 2 and 3
Goal	Evaluate the carbon footprint of Batch-ProA, Conti-ProA and Conti-PP
Functional unit	500 kg of mAb
Product demand	500 kg/year
Facility location	United Kingdom
Supply chain location	Europe
Impact categories assessed	Climate change (kg of CO_2 equivalent)
Evaluation metric	Product carbon footprint (kg of CO_2 equivalent)

The LCA boundary of this analysis was cradle-to-gate, i.e. from raw material extraction to waste disposal. It was essential to start at “the cradle” and include the emissions coming from supply chain activities so that the impact of different raw materials used in the flowsheets could be assessed. The study boundary ended at the “gate”, rather than “grave” aspect, as there was limited data on how the recycling of stainless-steel equipment is done across the industry or on how the product is discarded when it reaches the end of its useful life.

The carbon footprint of each flowsheet was divided into three categories: supply-phase, use-phase and end-of-life. The supply-phase considered raw material extraction, consumables and reagents production and the transport of these materials to the facility. The use-phase reflected the carbon footprint derived from the main production facility, including the energy and utilities required in the production process and in the facility in general. The end-of-life phase concerned the disposal of waste streams according to proper procedures.

The quantities of raw materials used, the processing times per unit operation and the weight of consumables waste were computed via the process economics model described in **Chapter 3**.

The LCA analysis is aligned with the Greenhouse Gas Protocol’s scopes 1, 2 and 3 for GHG emissions (described in **Section 1.5.3.2.1**). Scope 1 incorporated the entire manufacturing process and buffer and media preparation; Scope 2 involved the consumption of externally sourced energy, such as purchased electricity; and Scope 3 comprised the raw materials extraction and production, their transport and disposal. The equipment fabrication (usually included in Scope 1) was not included in the analysis, as its carbon footprint was assumed to be very small compared to other sources, due to the high durability of stainless-steel equipment and its frequent re-usage within the facility.

4.2.1 **Key assumptions**

4.2.1.1 Energy requirements

The unit operations included in each mAb production flowsheet require energy to run. Also, PW and WFI skids and ancillary activities, such as buffer and media preparation, involve electricity expenditures. These electricity requirements, including the energy needed for lighting the facility, were obtained from technical datasheets or gathered from the literature and are presented in
Table 4.2.

Table 4.2 - Energy input for the different unit operations and supporting activities used in mAb production process. Watt-units were multiplied by the time of operation. For media and buffer preparation, 1h of agitation was assumed. For lighting, the energy requirement was multiplied by the total site operating days.

Unit	Energy requirement	Reference
Cell Culture Agitation	2 W/L	(Doran, 2013)
Media/Buffer Preparation	70 W/L	(Walas, 1990)
Cell Culture Heating	7700 Wh	(AlfaLaval, 2022)
Centrifugation	17500 W	(ThermoFisher Scientific, 2021)
Filtration	50 W/m^2	(Lipnizki, Boelsmand and Madsen, 2002)
Chromatography	600 W	(Bunnak et al., 2016)
CIP/PW/WFI Generation	155 Wh/L	(Rawlings and Pora, 2009)
Lighting	15 W/m^2	(Bunnak et al., 2016)

The energy required for cell culture agitation can vary depending on factors such as the specific cell line, the stage of cell culture, the vessel type, and the agitation method used (Li et al., 2010). Specific power inputs (power/volume, P/V) for the agitation of CHO cells can range from 0.2 to 10 W/L (e.g., Balandras et al., 2011; Doran, 2013; Isailovic, Rees and Kradolfer, 2015) with

values more commonly reported at the lower end of the scale. For the analysis a value of 2 W/L (Doran, 2013) was selected.

Similarly, the level of agitation used in the dissolution of salts during media and buffer preparation also depends on the type of vessel and impeller. Values reported range from 0.5 – 70 W/L and the upper end was used in the analysis (Walas, 1990) to reflect the energy consumption in stirred tank reactors.

Besides the direct requirements of the bioprocess, there are other facility-related sources of energy consumption in biopharmaceutical facilities, including heat, ventilation and air conditioning (HVAC) systems. The energy requirements from the HVAC systems are dependent on the grade of each the cleanroom, based on the desired air change rate, and its floor area.

Table 4.3 shows the energy demand per cleanroom. These values were taken from Sinclair et al. (2008), who provided an overview of typical energy requirements for each area classification (Sinclair et al., 2008). As more recent data on the breakdown of these energy inputs per cleanroom were desired, a survey was sent to several partners in industry. However, it was not possible to gather a consistent range of values for the expected energy required per cleanroom class.

These parameters were later multiplied by each cleanroom area, derived from the process economics model according to **Section 4.2.1.1**. Also, the electricity needed for lighting all rooms in the facility was grouped with the HVAC requirements.

Table 4.3 - HVAC energy requirements per room classification (Sinclair et al., 2008).

Room	**Energy requirement (kWh/m2) on an annual basis**
Cleanroom C	237
Cleanroom D	119
Controlled Not Classified (CNC)	47
Unclassified area	47

The electricity source for the analysis was based on UK's national consumption mix of electricity, as documented in the EcoInvent database. The breakdown of this consumption mix is presented in the **Table 4.4**.

Table 4.4 - Electricity consumption mix in the United Kingdom from Ecoinvent database (2014).

Category	**Share of the Total Electricity Supply (%)**
Hydraulics	3
Nuclear	19
Fossil Fuel	60
Wind	9
Solar	1
Biowaste	9

4.2.1.1.1 Cleanroom classifications and facility area

The equipment footprint was calculated based on the area that each process skid occupied, according to each unit technical datasheet. For single-use hold buffer bags, these were stacked in piles of 2, 3 or 6, depending on the volumes. Bags larger than 1500 L were not stacked. Ranges of equipment footprints are shown in **Table 4.5**. The cleanroom area was determined assuming that the equipment occupied 15% of a cleanroom (Pereira Chilima, 2019) to allow for space for piping, walkways, maintenance and ancillary equipment, such as shelving, trolleys or testing devices.

Table 4.5 - Equipment footprint based on size range. This information was taken from the brochures of specific equipment.

Equipment	Size (min – max)	Floor Area (min – max) (m^2)
Bioreactor	10 – 25000 L	1.6 – 20
Hold vessel	10 – 20000 L	0.4 - 6.4
Bioreactor bag container	10 – 2000 L	1.2 – 10
Hold bag container	1 – 5000 L	0.1 – 1.6
Centrifuge	600 – 5000 L/h	3.5 – 4.5
Depth filter holder	1 – 24 m^2	0.2 – 2.1
Virus filter holder	1.5 – 10 m^2	0.1 – 0.3
ATF filter holder	0.13 – 11 m^2	0.1 – 0.3
Filtration skid	3 – 24 m^2	1.6 – 4.8
UFDF holder	1 – 100 m^2	0.1 – 0.5
UFDF skid	1 – 85 m^2	0.4 – 7.2
ILC & ILD module	0.13 – 1.2	0.2
Continuous VI tank	1 – 50 L	0.5 – 2.6
Chromatography skid (batch)	1 – 100 L/h	0.4 – 2.7
Chromatography skid (continuous)	1 – 6 L/h	0.9 – 3.5

The total facility footprint encompassed the main manufacturing area, clean circulation area, waste circulation, and the utility level and were determined based on the methodology provided in Pereira Chilima et al. (2016). However, certain zones were excluded from the facility footprint estimates. The general area, inclusive of the warehouse and logistics, was omitted due to the considerable variability in this estimation, which depends on each facility design. Additionally, areas such as Research and Development (R&D), Quality Assurance/Quality Control (QA/QC) and offices were not scoped in the study, as they can be separate from the main facility. **Table 4.6** shows the calculation factors to estimate the areas of the clean circulation, waste circulation and utility level based on the manufacturing footprint. The final facility areas were compared and validated against footprints provided by industry (personal

communication with Jasmin Kee, Kee Bio, UK) to increase the confidence in the HVAC calculations. Cleanroom classifications were based on information in the literature (Eibl and Eibl, 2019).

Table 4.6 - Cleanroom classification and facility footprint calculating ratios for stainless-steel (SS) and single-use (SU) based flowsheets (Pereira Chilima et al., 2016). In the manufacturing area, 16 m^2 are added to each room to account for the airlocks.

Area	Description	Ratio of manufacturing footprint	
		SS based	SU based
Manufacturing	Classification C • Cell culture – seed • Polishing (CEX, AEX, VF, UFDF, ILC, ILD) Classification D • Cell culture – main • Centrifugation • Depth Filtration, capture, VI Classification CNC • Buffer preparation	1	1
Clean circulation	Classification CNC • Clean corridors • Buffer storage • Operator stations	0.2	0.2
Waste circulation	Classification CNC • Waste corridors • Staging	0.2	0.2
Utility level	Classification U • CIP • PW/WFI	1.2	1

4.2.1.2 Database

As aforementioned, EcoInvent 3.7 was the main LCA database used in this study. However, items of various streams, including reagents and consumables, were not available in the database. Thus, some approximations and suitable substitutions to specific components available in the database had

to be made. The main assumptions regarding the database of raw materials are listed below.

- Cleaning solutions

  In the CIP buffer, sodium hydroxide was assumed as the main component;

- Cell culture media

  The media individual components were taken from technical datasheets of media from Lonza Pharmaceuticals;

- Chromatography buffers

  Protein A chromatography buffers were based on the buffers used by Pollock et al. (2013) in the application of semi-continuous chromatography for commercial manufacture (Pollock et al., 2013). The CEX and AEX buffers were obtained from Cytiva. The buffers were mostly composed of Tris buffer and NaCl. Tris was substituted by the closest analogue in EcoInvent database, dimeethylaminopropylamine;

- Viral inactivation buffer

  For simplification, it was assumed that citric acid was the major component in this reagent recipe;

- Plastic consumables and resins

  The carbon footprint of the consumables of each stream (i.e., SU bags, filters and pre-packed columns) was derived from Ramasamy (2018) using linear regressions according to the specified size of each item. The items and respective carbon footprint used in the regressions are shown in **Table 4.7**. As there was no data on the carbon footprint of fabricating chromatography pre-packed columns, a simplification was made and it was assumed the same kg CO2 per kg as in the filters. The calculated carbon footprint included raw material extraction of the required components, molding or fabricating to the final form, sterilisation and delivery of the items to the facility (Ramasamy, 2018).

- Liquid waste treatment

  The liquid waste was assumed to be heat treated in-house before being sent for disposal;

- Liquid and solid waste disposal

  The single-use materials (solid waste) were assumed to be disposed via incineration, while the liquid waste was transported to the local wastewater treatment facility.

Table 4.7 - Carbon footprint of different consumables based on size (Ramasamy, 2018).

Item	Size	Carbon footprint (CO_2-eq)
SU bag	10 L	35
	200 L	60
	500 L	126
Filter	0.3 kg	8
	1 kg	45
ProA resin	-	77 CO_2-eq/kg
AEX & CEX resin	-	18 CO_2-eq/kg

4.2.1.3 Flowsheets optimisation

The impact of process changes in the environmental footprint of mAb production flowsheets was assessed. Discussions with the Przybycien group from Renssealaer Polytechnic Institute, who published on the precipitation conditions taken as reference for this thesis on mAb production with capture by precipitation (Li et al, 2019), revealed that critical process parameters have been optimised to reduce water and raw materials consumption since the work outlined in **Chapter 3**. These changes were introduced in the process economics model and the outputs were compared with the product carbon footprint from the base case. Also, increased titres and volumetric productivities

were scoped in during the evaluation of the flowsheets environmental impact. **Table 4.8** shows the parameters that were changed in each one of the flowsheets during the environmental assessment.

Table 4.8 - Process parameters changed in Batch-ProA, Conti-ProA and Conti-PP optimisation.

Flowsheet	Parameter	Base case	Best case
Batch-ProA	Cell culture titre	5 g/L	15 g/L
	Equivalent volumetric productivity	0.5 g/L/day	1.25 g/L/day
Conti-ProA	Perf. Volumetric Productivity	3 g/L/day (2 g/L harvested titre)	9 g/L/day (6 g/L harvested titre)
Conti-PP	Perf. Volumetric Productivity	3 g/L/day (2 g/L harvested titre)	9 g/L/day (6 g/L harvested titre)
	Concentration step prior PP	None (2 g/L harvested titre)	Up to 5-fold (10 g/L harvested titre)
	HCCF %	50%	80%
	Capture yield	74%	90%

From discussions with the Przybycien research group, the best titre envisioned for the future of fed-batch mAb manufacture was 15 g/L (equivalent volumetric productivity of 1.25 g/L/day). For the fed-batch and perfusion stages, the volumetric productivity difference was kept constant (at 7.2-fold) for the base case (0.42 vs 3.0 g/L/day) and best cases (1.25 vs 9 g/L/day). Therefore, the best case titre for perfusion was at 6 g/L.

In Conti-PP, the base case was based on data provided in Li et. al (2019) and the best case was based on unpublished data from the Przybycien research group. The best case assumed an HCCF concentration increase from 2g/L to 10 g/L through the implementation of a filtration step prior to the precipitation based on proof-of-concept currently under development by Przybycien's group. This concentration step was implemented without hampering the precipitation

step (acceptable liquid viscosity). Also, conversations with the same group revealed that the 2-fold dilution of HCCF seen in Conti-PP base case (50% HCCF in the precipitation system) could be reduced through the increase of the HCCF ratio in the precipitation system (from 50 to 80% HCCF) with the utilisation of higher concentrated stock solutions of PEG and zinc chloride and lower quantities of PEG (%PEG was reduced from 7 to 5% after process optimisation). Additionally, major advances concerning mAb recovery from the precipitates wash step were shown during these conversations, which allowed for an overall capture yield increase of more than 20% (capture yield base case=74%; capture yield best case=90%).

4.2.1.4 Benchmarks of carbon emissions

The product carbon footprint of each flowsheet was translated into day-to-day metrics to allow for a more relatable understanding of their environmental impact.

Table 4.9 shows the approximated conversion rates from 1 ton of emitted CO_2 into several metrics, with the respective assumptions.

Table 4.9 - Conversion rates for the emissions of 1 ton CO_2-eq.

Metric	Assumption	Conversion rate 1 ton CO_2-eq	Reference
Number of individuals equivalent emissions	• 5000 kg CO_2/person/year	0.20	(Statista, 2023a)
Number of intercontinental flights	• London to New York • 5 000 km • 0.15 kg CO_2/passenger/km • 400 passengers	0 3	(Statista, 2023b)
Number of trees to offset emissions	• 22 kg CO_2 absorbed/tree/year	46	(Encon, 2023)

4.3 Results and discussion

The environmental outputs of producing monoclonal antibodies using protein A chromatography or product precipitation for capture were derived by combining the decision-support tool and OpenLCA. In this section, the sustainability assessment started by diving into the product carbon footprint of Batch-ProA, Conti-ProA and Conti-PP, which was compared with the PMI metric derived in Chapter 3. The breakdown of energy requirements and carbon footprint of single-use items was subsequently showed to provide insights on the total product carbon footprint trends. The last stage of analysis comprised the optimisation of the flowsheets, with focus on Conti-PP as a column-free capture alternative, to identify the key changes in process parameters that could lead to a more environmentally friendly production scheme. All studies were performed for a mAb demand of 500 kg/year.

4.3.1 Product carbon footprint

After integrating the mass balances of each process together with the calculated energy requirements and SU consumables carbon footprint directly in OpenLCA, the lifecycle assessment of Batch-ProA, Conti-ProA and Conti-PP was performed.

The PMI analysis presented in Chapter 3 indicated that continuous flowsheets result in lower PMI metrics that focus on waste generation. Here the analysis explored whether continuous flowsheets would also result in lower carbon footprints. **Figure 4.1** presents the product carbon footprint (PCF) of each flowsheet, which corresponded to the climate change impact category from OpenLCA. The results indicated that whilst Conti-ProA can lead to savings in PCF, Conti-PP led to the highest carbon footprint from all three flowsheets (PCF: 4.8×10^5 kg CO_2/year Batch-ProA; 3.6×10^5 kg CO_2/year Conti-ProA; 7.1×10^5 kg CO_2/year Conti-PP). This is in contrast to the rankings observed when using the PMI metric for waste in **Section 3.3.2**, where both continuous options (column-based and column-free) resulted in significantly less waste and hence lower PMI metrics.

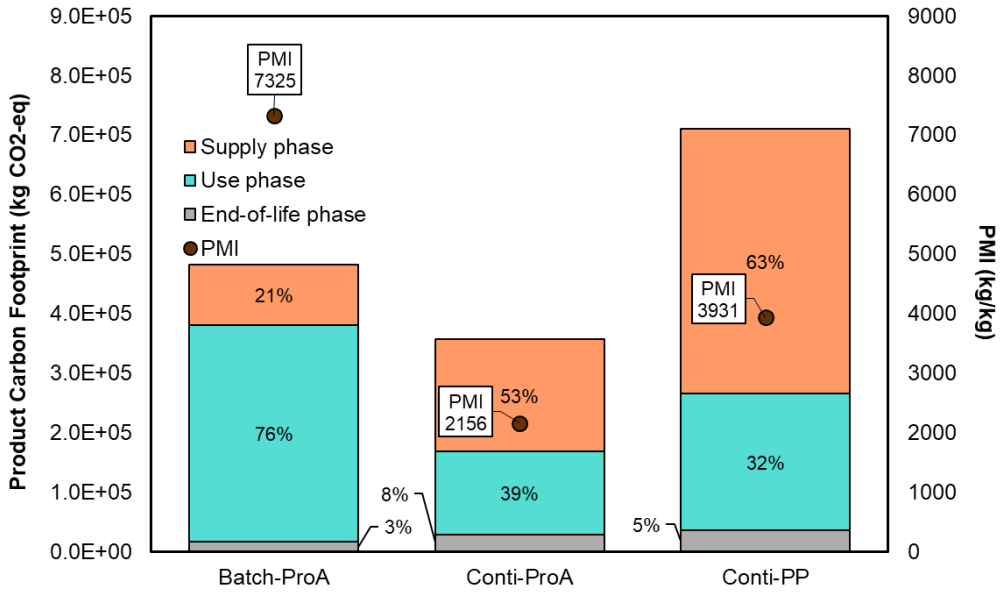


Figure 4.1 - Product carbon footprint and PMI for an annual demand of 500 kg/year using Batch-ProA, Conti-ProA or Conti-PP flowsheets.

The investigation and comparison of the PMI and PCF metrics revealed that they yield different conclusions owing to the different environmental impacts that they capture. Since the PMI metric focuses on waste generation and biotech processes use more water than consumables, it will favour process intensification strategies such as continuous that reduce water consumption. The PMI treats the consumables and reagents streams with equal weighting and does not account for the specific fabrication process of the items. In contrast, since the PCF metric focuses on climate change, it will favour processes with lower GHG emissions.

The breakdown of the PCF was analysed to determine the key drivers for each flowsheet and to determine why the Conti-PP led to the highest carbon footprint. As described in **Section 4.2**, the PCF was divided into 3 categories: supply-phase, use-phase and end-of-life. The main driver for the high carbon footprint of Conti-PP was the supply-phase related emissions for extracting and producing the raw materials (63%). This exceeded the carbon footprint related to the energy requirements (use-phase) (32%) or disposing the waste streams after production (end-of-life phase) (5%). The carbon footprint from the supply-phase in Conti-PP was, approximately, 4 and 2-fold higher than Batch-ProA and Conti-ProA, respectively (supply-phase carbon footprint: 1.0×10^5 kg

CO_2/year Batch-ProA; 1.9x10^5 kg CO_2/year Conti-ProA; 4.5x10^5 kg CO_2/year Conti-PP). For Batch-ProA, the main carbon footprint contributor was the use-phase, which accounted for 76% of the total PCF. The use-phase in Batch-ProA was 2.6 and 1.6-fold higher than Conti-ProA and Conti-PP, respectively, mainly due to the large energy requirements from CIP buffer generation (use-phase carbon footprint: 3.6x10^5 kg CO_2/year Batch-ProA; 1.4x10^5 kg CO_2/year Conti-ProA; 2.3x10^5 kg CO_2/year Conti-PP). Similarly to Conti-PP, the supply-phase emissions also dominated Conti-ProA's PCF (53%), while the use-phase comprised 39% of the flowsheet related emissions. The reduced use-phase emissions compared to Batch-ProA and Conti-PP contributed to the lowest Conti-ProA's PCF value amongst flowsheets.

The detailed breakdowns of the GHG emissions related to the supply, use and end-of-life phases from each flowsheet are explored in the following sections to help explain the contributing factors to the trends.

4.3.2 Carbon footprint of the supply-phase materials

Error! Reference source not found.**a** shows the breakdown associated with the carbon emissions from the supply phase for each flowsheet and the contribution of consumables and reagents components fabrication. Error! Reference source not found.**b-e** present the contribution per type of reagent or consumable and per production stage (USP/capture/polishing). As described in the previous section, continuous flowsheets presented higher supply-phase emissions than in batch (1.9 and 4.4-fold higher for Conti-ProA and Conti-PP, respectively). Additionally, for these flowsheets, consumables production dominates the supply-phase emissions, while in batch the contribution of consumables and reagents production is almost equal (ratio of consumables and reagents production: 44:56 Batch-ProA; 87:13 Conti-ProA; 68:32 Conti-PP). These results help highlighting trends between stainless steel based (Batch-ProA) and single-use based (Conti-ProA and Conti-PP) facilities. The consumables emissions relate to the fabrication of SU bags, resins and filtration membranes, while the fabrication of reagents components includes salts used in culture

media and buffers and specific chemicals, like PEG, used in the precipitation of antibodies in Conti-PP.

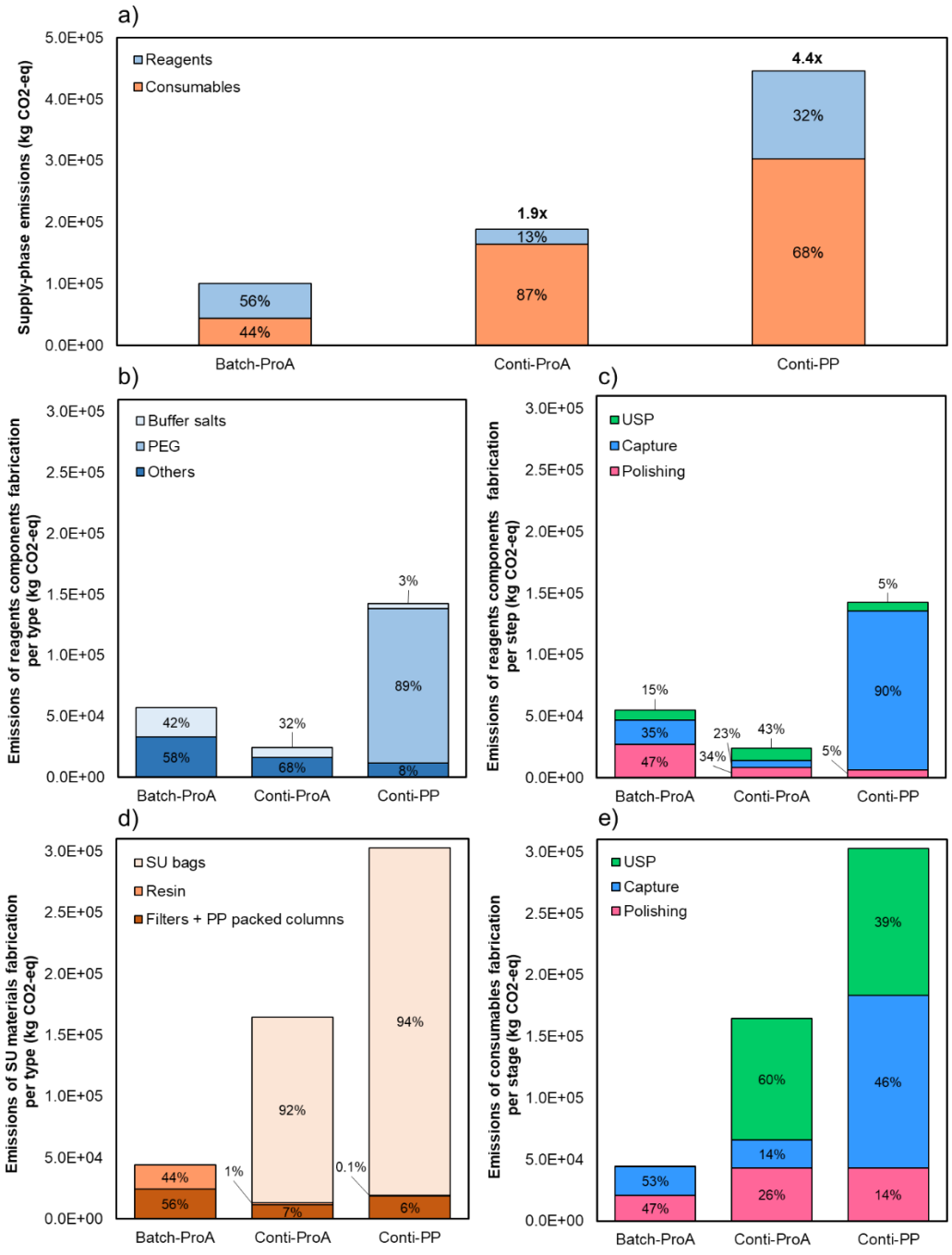


Figure 4.2 - Breakdown of a) carbon emissions related to the entire supply-phase and fabrication of b) reagents per type, c) reagents per production stage, d) consumables per type and e) consumables production stage for an annual demand of 500 kg/year using Batch-ProA, Conti-ProA or Conti-PP flowsheets. The polishing steps' emissions are the same for Conti-ProA and Conti-PP, but represent different percentages of the emissions.

Conti-PP presents the highest supply-phase emission, with both consumables and reagents fabrication higher than Batch-ProA and Conti-ProA. The emissions derived from consumables fabrication are 6.8 and 1.8-fold larger than in Batch-ProA and Conti-ProA, respectively, and the emissions from reagents are 6.0 and 2.5-fold larger than in Batch-ProA and Conti-ProA, respectively.

The significant contribution (89%) of PEG to the reagents emissions of Conti-PP is showed in Error! Reference source not found.**b** and explains the major impact of the capture stage in the flowsheet reagents emissions (Error! Reference source not found.**c)**. Moreover, the emissions from PEG fabrication represent 9% of the total PCF from Conti-PP. The production of polyethylene glycol is derived from ethylene oxide, which is produced from ethylene, a hydrocarbon derived from fossil fuels. Also, the polymerisation process into PEG requires high temperatures and pressures, leading to increased energy consumption. This results in a carbon-intensive raw material that is also used in a significant amount in Conti-PP. Sodium chloride, which is used in the culture media, chromatography buffers or antibody precipitation, has also a visible contribution in the reagents emissions, especially in Batch-ProA (contribution of sodium chloride in the reagents emissions: 42% in Batch-ProA; 32% in Conti-ProA ; 3% in Conti-PP).

The impact from the fabrication of different consumables is showed in Error! Reference source not found.**d** and reveals that SU bags in Conti-ProA and Conti-PP drive the high consumables emissions from these continuous flowsheets (>90% contribution). In Conti-PP, the number of single-use bags used in the capture stage is significantly larger than Conti-ProA, which supports the higher consumables emissions from this stage (Error! Reference source not found.**e)**. This can be attributed to the 4-fold higher number of larger (>= 1000 L) SU bags used in the capture stage when precipitation is used instead of ProA.

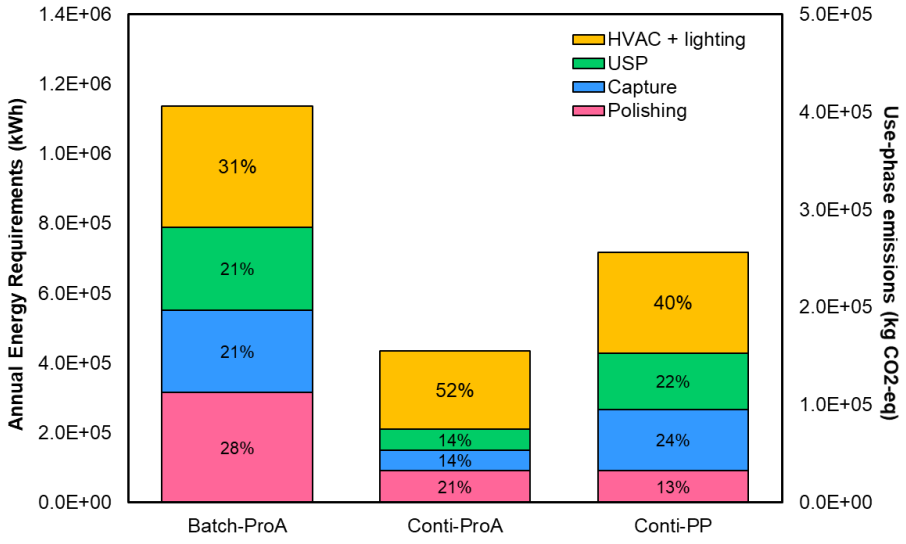
The consumables emissions presented in Batch-ProA derive only from the production of UFDF filters, guard filters and resins. As expected from the PMI analysis, the consumables environmental impact in a stainless-steel based facility are significantly lower than in a single-use based one.

4.3.3 Carbon footprint of the use-phase: Energy requirements

The deeper analysis of the energy requirements provided clarification on the use-phase carbon footprint shown for each flowsheet. **Error! Reference source not found.** depicts the breakdown of the annual energy requirements by major processing stage (USP/capture/polishing) combined with the HVAC and lighting energy consumption.

This investigation highlighted that Batch-ProA had the highest energy consumption out of all the flowsheets. This can be attributed to both the larger scale required in batch and the need for CIP with stainless steel equipment. This is reinforced by the larger manufacturing area (embedded table) that directly impacts the HVAC and lighting consumption and the high contribution of emissions coming from CIP buffer generation. From Chapter 3, it was clear that the CIP cleaning in Batch-ProA led to a negative impact both on costs and PMI. In the analysis showed in **Error! Reference source not found.**, it can also be confirmed that stainless-steel based facilities will require an increased energy input for CIP buffer preparation (CIP generation represents 22% of the total energy requirements – embedded table). This is also reflected in the large energy values and high contribution of the polishing stage ($3.2x10^5$ kWh/year, 28% of total annual consumption), which was the stage with more unit operations and, therefore, more CIP buffer required.

Turning the comparison to the continuous flowsheets, while both Conti-ProA and Conti-PP presented lower energy requirements than Batch-ProA (annual energy requirements: $1.1x10^6$ kWh/year Batch-ProA; $4.3x10^5$ kWh/year Conti-ProA; $7.2x10^5$ kWh/year Conti-PP), Conti-ProA was clearly the less energy-intensive option studied.



Equipment footprint (m²)	105	60	94
Total manufacturing area (m²)	797	550	726
% HVAC & lighting in total requirements	31%	52%	40%
% media & buffer prep in total requirements	7%	17%	14%
% WFI generation in total requirements	26%	22%	40%
% CIP generation in total requirements	22%	-	-
% unit operation energy in total requirements	15%	9%	5%
Breakdown of energy requirements - CAPTURE			
Depth Filtration	43%	-	-
Centrifugation	0%	-	-
Protein A chromatography	57%	100%	-
Precipitation	-	-	69%
Concentration & wash	-	-	19%
Resolubilisation	-	-	6%
Precipitates depth filtration	-	-	6%
Inline concentration	-	-	<1%
Breakdown of energy requirements - POLISHING			
Virus inactivation	3%	5%	
CEX	23%	25%	
AEX	22%	17%	
Virus Filtration	3%	25%	
UFDF	49%	-	
ILD	-	1%	
ILC	-	28%	

Figure 4.3 - Breakdown of energy requirements for an annual demand of 500 kg/year using Batch-ProA, Conti-ProA or Conti-PP flowsheets. The total energy requirements of Conti-ProA and Conti-PP are benchmarked against Batch-ProA. SIP was not considered in the process economic model, therefore, the energy from steam generation is not included in product carbon footprint. Media and buffer prep row relate to the energy usage during mixing of the ingredients. WFI and CIP generation rows indicate the energy spent in the stations used to prepare these liquids. The polishing steps energy requirements are the same for Conti-ProA and Conti-PP, but represent different percentages of the total energy requirements.

As observed in **Figure 3.1**, Conti-ProA presented the shortest length overall and for the capture train specifically, which had a direct impact on the total facility footprint and resulted in lower HVAC and lighting requirements. Moreover, the energy needed to prepare buffers and run the unit operations in the capture stage came uniquely from the protein A chromatography step. In contrast, Conti-PP required higher energy consumption for HVAC and operations due to the 5-step capture train and the higher buffer demand from the concentration, wash and resolubilisation steps during the capture stage. Also, as discussed in the PMI analysis in **Section 3.3.2**, due to the lower DSP yields, more product output was needed from Conti-PP's cell culture to meet the same annual demand and, thus, more media was required.

While the PMI metric only showed the larger media volumes needed in Conti-PP compared to Conti-ProA, the bar chart in **Error! Reference source not found.** revealed the big impact that this also had on the facility energy requirements. The energy contribution from the USP step in Conti-PP is almost 3-fold higher than in Conti-ProA (USP annual energy requirements: $2.4x10^5$ kWh/year Batch-ProA; $6.0x10^4$ kWh/year Conti-ProA; $1.6x10^5$ kWh/year Conti-PP).

The contributions of each stage (USP/capture/polishing) or category (e.g., HVAC, buffer preparation, unit operations) on the total energy requirements will highly depend on the energy input (kWh) of the utilities and unit operations, as well as on the clean rooms' classifications in a certain facility. HVAC, for instance, is used to ensure the optimal conditions in production rooms by regulating temperature and humidity. Thus, it is expected to contribute a significant portion of the energy usage in a facility. However, its actual impact will depend on several parameters, such as the type of fan filter units utilised, the choice of heating/cooling equipment and the air change rates (total air volume in a certain space that is replaced with fresh or recirculated air) per cleanroom to meet the required concentration of particles according to GMP standards. As more HVAC energy data becomes available in future and more efficient HVAC systems are designed, it is expected that these differences would have an impact on the energy utilisation reported.

In the present study, the HVAC contribution to the total energy usage of mAb facilities ranged from 30 to 50%, depending on the production scheme. Additionally, when looking into the HVAC energy required per square meter, the flowsheets in this thesis presented consumptions ranging from 400 to 450 kWh/m^2 (Sinclair et al., 2008). Other studies (e.g., Galitsky, 2011; Ramasamy, 2018) used benchmark values for HVAC consumption 5 to 10-fold higher than Sinclair (2008) (Boyd, 2005; Capparella, 2013), which led to HVAC contributions from 65 to 85% of the total energy requirements. However, these benchmarks did not consider the different HVAC features per cleanroom and there was limited information on the scale of production or the area classifications included in the HVAC area. Once more, it is important to acknowledge that the energy outputs presented in this section were based on the references showed in

Table 4.2 and **Table 4.3** and that other assumptions may yield different outcomes. Discussions with industry experts also revealed that a wide range of energy usage can be expected from facilities with different configurations and strategies. Therefore, the total product carbon footprint will also be influenced from facility-to-facility or scenario-to-scenario, yielding to higher or lower values than the ones reported in this thesis.

4.3.4 Carbon footprint of end-of-life phase

The emissions from the end-of-life phase relate to the activities of disposing the waste streams from each production flowsheet.

Although in Batch-ProA the liquid waste stream is significantly higher than in the continuous flowsheets (**Figure 4.1**), the disposal of solid waste in Conti-ProA and Conti-PP drove the higher carbon footprint of the end-of-life phase (end-of-life carbon footprint: $1.6x10^4$ kg CO_2/year Batch-ProA; $2.9x10^4$ kg CO_2/year Conti-ProA; $3.6x10^4$ kg CO_2/year Conti-PP). The contribution of the end-of-life phase for the total PCF was lower than 10% amongst flowsheets; however, this may vary according to the assumptions taken for liquid and solid disposable in OpenLCA. In the present study, the solid waste was treated via incineration and the liquid waste was sent to the local wastewater treatment

facility for treatment. These options were discussed with industrial partners and recognised as the most common practices currently applied in the sector.

4.3.5 Optimisation of batch and end-to-end continuous mAb production

As discussed in **Chapter 3**, there have been efforts from both academia and industry towards improving the cell culture output in mAb manufacture. These improvements in mAb titres lead not only to economic advantages but can also reflect a significant decrease in the environmental impact in mAb flowsheets.

Figure 4.4 shows that Conti-PP is the flowsheet that benefited the most from the increase in cell culture productivity. While Batch-ProA and Conti-ProA presented PCF reductions of 8 and 22%, respectively, the carbon footprint of Conti-PP was 50% lower moving from 3 g/L/day to 9 g/L/day.

For the same annual demand (500 kg/year), a more concentrated cell culture fluid results in a smaller volume output. Thus, the 3-fold increase in mAb titre (Batch-ProA) or volumetric productivity (Conti-ProA and Conti-PP) had a direct impact on the USP and DSP sizes. In Conti-PP, the capture steps (e.g., precipitation, concentration) were sized based on the HCCF volume, whereas in Batch-ProA and Conti-ProA the chromatography step was sized based on the product mass (the quantity of mAb in the HCCF is the same regardless the HCCF titre). As a result, the decrease in HCCF volume through the increase in perfusion productivity had a more significant impact in the carbon footprint of Conti-PP.

The carbon footprint related to the supply-phase, use-phase, and end-of-life before and after simulating the optimisation in titre in Batch-ProA is nearly the same. In Conti-ProA, the lower supply-phase footprint is the driver for the PCF reduction, due to the decrease in media consumption and SU bags in the USP. In Conti-PP, both supply-phase and use-phase showed a strong decrease of 56 and 42%, respectively, mainly caused by the decrease in media, buffer, and PEG volumes and the associated decrease in energy required for material preparation.

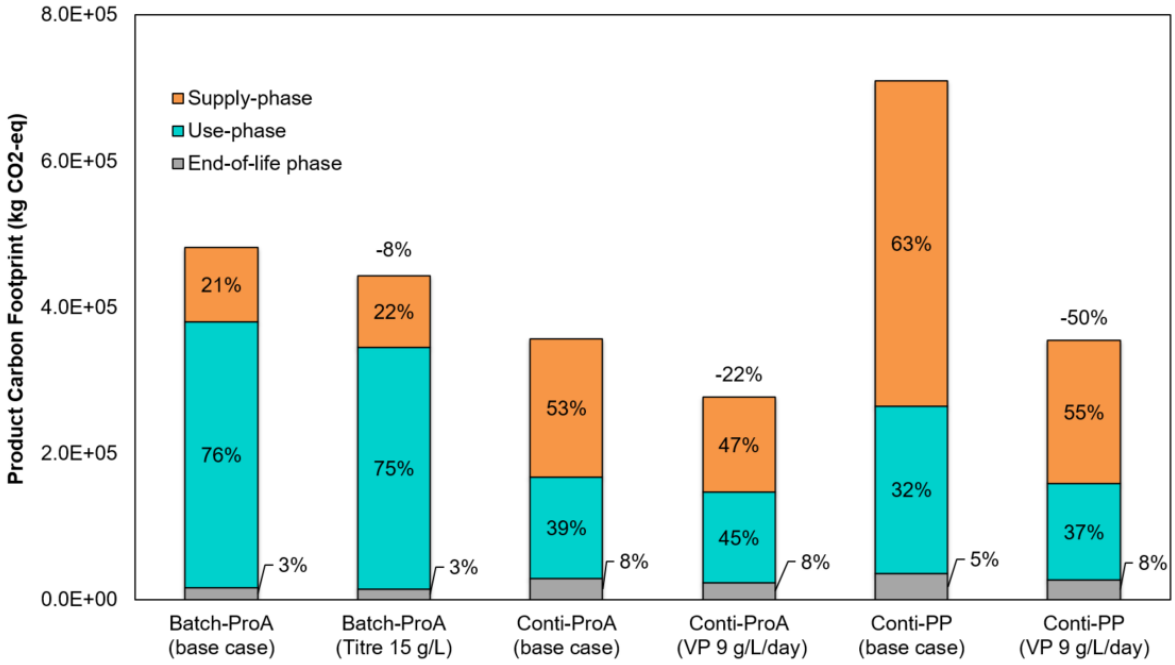


Figure 4.4 - Comparison of product carbon footprint obtained for the base-case and optimised Batch-ProA, Conti-ProA or Conti-PP flowsheets for an annual demand of 500 kg/year using.

Table 4.10 portrays the key drops in output parameters from optimising the perfusion volumetric productivity in Conti-PP. The major decrease in reagent volumes and resulting decrease in SU hold bags led to a 40% smaller facility area. As a result, the HVAC and lighting requirements, which comprised 40% of the total energy requirement in Conti-PP's base-case, were reduced in 39%. In the supply-phase, the SU materials and PEG emissions decreased 53 and 67%, respectively.

4.3.5.1 Strategies to decrease the product carbon footprint of Conti-PP

In addition to increasing cell culture productivity, the environmental impact of improving other DSP-related features in Conti-PP was investigated. The collaborative discussions with Przybycien's research group that formed the basis of Conti-PP flowsheet uncovered specific advances in several capture steps, as described in **Section 4.2.1.3**. Ultimately, the investigators suggested that these improvements could be combined into an optimised Conti-PP flowsheet with a decreased product carbon footprint.

Table 4.10 - Key environmental outputs and respective reduction for Conti-PP before and after process optimisation.

Output	Base Case Conti-PP	VP 9 g/L/day	Reduction
PMI (kg/kg)	3 931	1 944	-51%
Use-phase			
Equipment footprint (m^2)	94	51	-46%
Total manufacturing area (m^2)	1 742	1 039	-40%
Annual HVAC + lighting requirements (kWh)	289 048	175 760	-39%
Total annual energy requirements (kWh)	716 392	412 680	-42%
Supply-phase			
Annual SU emissions (kg CO_2-eq)	302 777	142 719	-53%
PEG carbon footprint (kg CO_2-eq)	127 000	42 220	-67%
Total product carbon footprint (kg CO_2-eq)	1 415 217	393 687	-50%

This section aims to provide insights on the strategies that lead to the most accentuated PCF decrease in Conti-PP and highlight the priorities for process optimisation. **Figure 4.5** shows that increasing the volumetric productivity (VP) in cell culture is still the strategy with the highest impact on product carbon footprint (-50%). This strategy reduces media consumption and the HCCF volume, as previously discussed. The volume of media in cell culture has a direct impact on the energy required for media production and on consumables emissions from SU hold bags fabrication. On the other hand, reducing the HCCF % influences the volume of PEG needed. Comparing to the VP increase, the integration of a mAb concentration step to 10 g/L before the product precipitation resulted in a higher reduction in HCCF volume before precipitation (reduction of HCCF volume compared to base-case: 3-fold with increased VP; 5-fold with concentration step) which led to an 80% decrease in PEG emissions; however, there was no impact on the required media volumes. The integration of a concentration step led to a PCF decrease of 37%.

The strategies of increasing the HCCF percentage in the precipitation system or increasing the overall capture yield had a similar effect on the PCF reduction from the Conti-PP base-case (-26% and -19%, respectively). According to the Przybycien's research group, changing the HCCF% from 50 to 80% was possible by optimising the quantity of PEG required for precipitating the product (%PEG reduced from 7 to 5%) and utilising higher concentrated stock solutions of PEG and zinc chloride. This had a direct impact not only on the PEG footprint (-55%), but also on the SU bags required in the following steps and (-26% consumables emissions). Increasing the overall capture yield was possible through the efforts shown by academia on optimising the precipitates wash step. The increase in capture yield from 74 to 90% led to a smaller USP and DSP size, with modest effects on the energy requirements (-16%), consumables emissions (-24%) and PEG carbon footprint (-17%).

All process improvements described in **Figure 4.5** were sequentially implemented by order of impact in reducing the product carbon footprint of Conti-PP. **Figure 4.6a** shows the cumulative influence of implementing the process changes in terms of energy and PEG consumption, while **Figure 4.6b** presents the overall PCF decrease in Conti-PP.

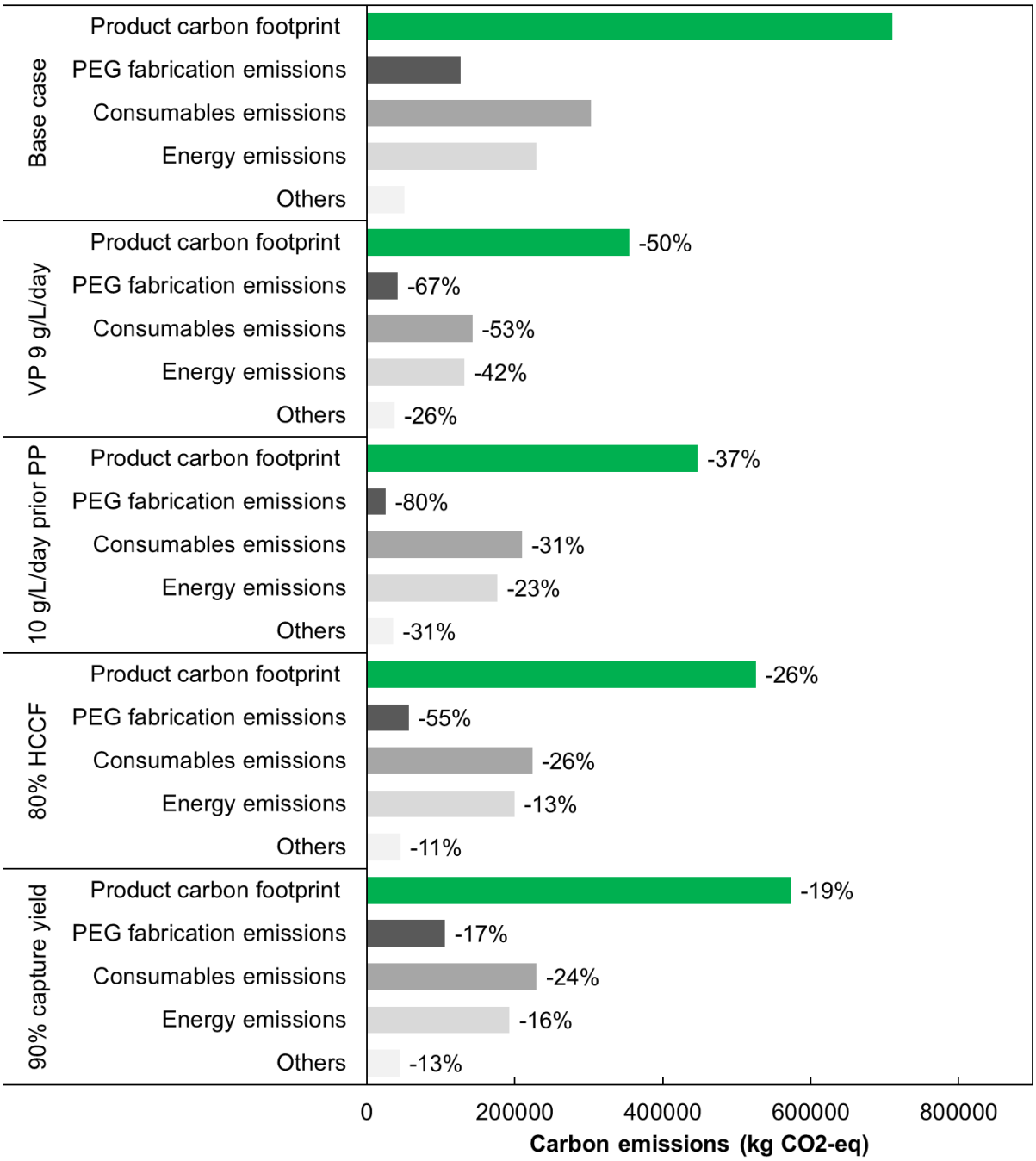


Figure 4.5 - One-way sensitivity analysis from implementing different process improvements in Conti-PP. All percentage differences refer to the parameters' values found for Conti-PP base-case. "Others" refer to all supply-phase related emissions besides PEG and consumables (e.g., salts) and the end-of-life phase emissions.

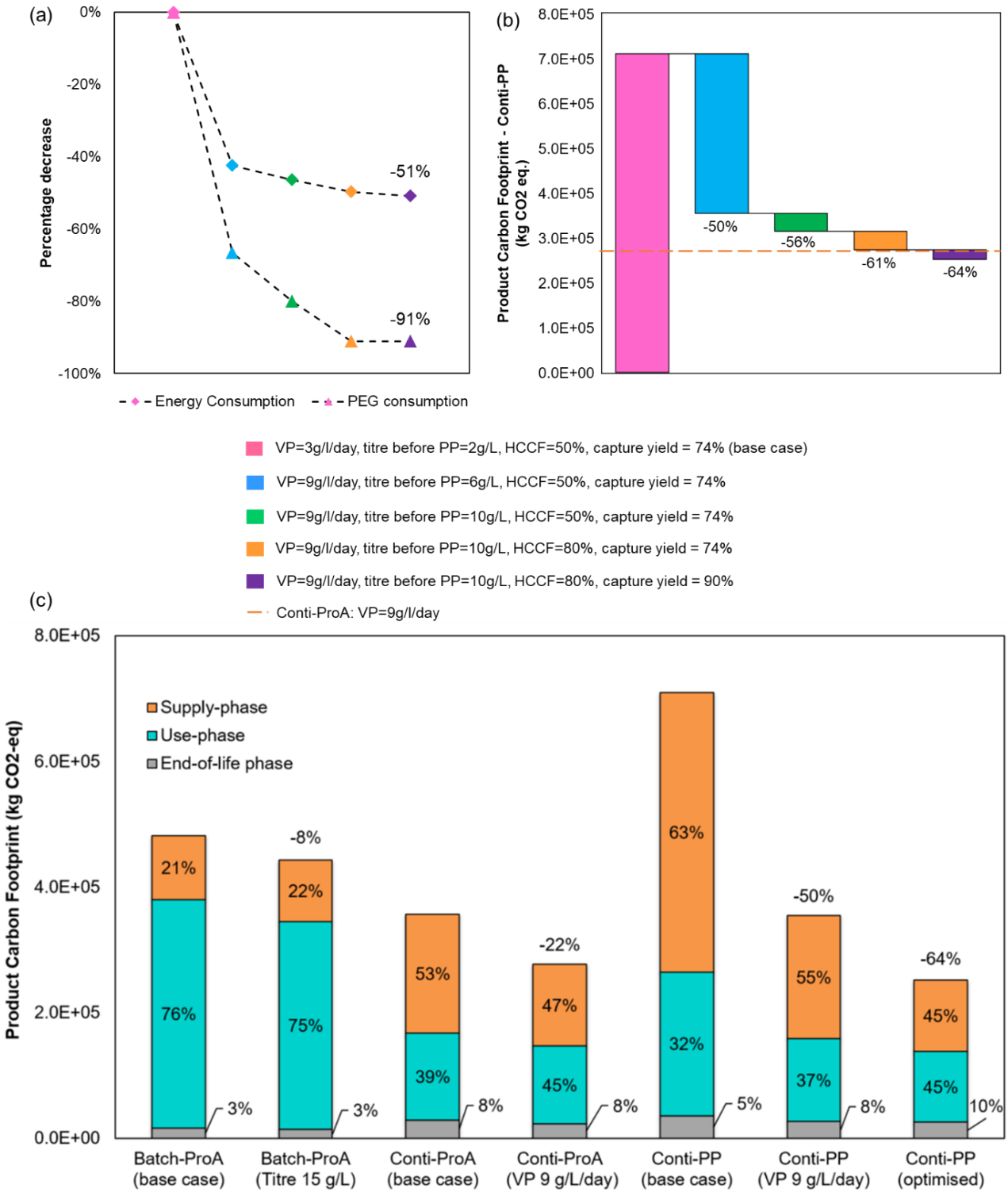


Figure 4.6 - a) Energy and PEG consumptions and b) product carbon footprint reduction after implementation of sequential process optimisations in Conti-PP. c) Comparison of product carbon footprint obtained for the base-case and all optimised Batch-ProA, Conti-ProA or Conti-PP flowsheets. All results are showed for an annual demand of 500 kg/year. In c) “optimised” refers to the implementation of all USP and DSP improvements.

Savings in energy consumption increased from 42 (with a perfusion volumetric productivity of 9 g/L/day) to 51% after integrating all remaining DSP improvements. The decrease in PEG consumption was more accentuated after a complete optimisation, as the PEG quantity required in the process could be reduced up to 91% if all advances were to be integrated.

The full extent of process optimisation in Conti-PP could lead to a PCF decrease of 64%, as shown in **Figure 4.6b** and **Figure 4.6c** also shows an even contribution of the supply and use phases for the total product carbon footprint, as PEG fabrication emissions are significantly reduced (reduction of supply-phase contribution). The 64% savings in PCF represented a modest extra 14% improvement comparing to the PCF reduction attained after improving only the cell culture productivity.

The estimated PCF value after the complete optimisation of Conti-PP was, approximately, 9% lower than Conti-ProA's optimised flowsheet with a volumetric productivity of 9 g/L/day (PCF after optimisation: 2.7×10^5 kg CO_2-eq Conti-ProA; 2.5×10^5 kg CO_2-eq Conti-PP). As seen in **Section 3.3.6**, the effect of increasing the perfusion volumetric productivity is higher in Conti-PP's COG than in Conti-ProA. Thus, further economic and PCF reductions might also entail putting efforts into boosting the USP output in mAb manufacture to help the business case of Conti-PP.

4.3.6 Benchmarks of mAb product carbon footprint

Converting the values calculated for the carbon footprint of each flowsheet into more tangible day-to-day metrics allowed for a more relatable understanding of its environmental impact. Travel distances (e.g., intercontinental flights) or land area (e.g., hectares of trees) are relatable measurements that can help to grasp the scale of carbon emissions in this study. The impact of optimising Batch-ProA, Conti-ProA and Conti-PP flowsheets is presented in **Table 4.11**.

Table 4.11 shows that, after process optimisation, Batch-ProA is the flowsheet whose PCF is translated into the largest day-to-day carbon metrics, while Conti-ProA is the strategy with the lowest values amongst all.

Table 4.11 - Conversion of Product Carbon Footprint into day-to-day activities and annual carbon emission taxes in the UK. All metrics are calculated on a yearly basis and relative to the PCF values for a demand of 500 kg mAb/year.

	Base-case			**Optimised flowsheet**		
	Batch ProA	**Conti ProA**	**Conti PP**	**Batch ProA**	**Conti ProA**	**Conti PP**
PCF (kg CO_2-eq) (annual basis)	$4.8x10^5$	$3.6x10^5$	$7.1x10^5$	$4.4x10^5$	$2.7x10^5$	$2.5x10^6$
Number of individuals equivalent emissions	97	72	143	89	56	51
Number of intercontinental flights	2	2	3	2	1	1
Number of trees to offset emissions (hectares[a]/ stadiums[b])	22 167 (56/112)	16 416 (42/84)	32 682 (82/164)	20398 (51/102)	12 740 (32/64)	11 614 (30/60)

Note: a) 400 trees/hectare; b) 0.5 hectares/stadium

As demonstrated in the previous section, the optimisation of Conti-PP resulted in 64% PCF savings. To put this into a broader context with day-to-day carbon metrics, this was found to be equivalent to the decrease in 92 individuals' emissions, 2 flights from NY to London or 21068 trees to offset emissions (equivalent of 104 hectares stadiums). Especially turning the attention to the savings in trees to offset emissions, it was possible to understand the significance of the outputs from this chapter. Additionally, highlighting the equivalence of PCF reductions to these tangible carbon savings emphasised the positive environmental impact of the optimisation efforts.

4.4 Conclusions

The life cycle assessment of mAb production using different flowsheets provided a deeper understanding of its environmental impact. The evaluation was conducted for an annual mAb demand of 500 kg/year and encompassed energy requirements, carbon footprints, and compared the environmental output before and after the optimisation of each flowsheet. Previous indications from the Process Mass Intensity (PMI) evaluation showed that the stainless-steel based batch production was the least environmentally friendly from all flowsheets; nevertheless, the single-use based continuous flowsheet with product precipitation emerged as the option with the highest product carbon footprint (PCF) among the strategies after a life cycle assessment. This emphasised that PMI and PCF metrics can result in different conclusions, as the first focuses on resources consumption (e.g., water and consumables) and the second focuses on processes' GHG emissions. The preferred metric to focus on will depend on each company's strategies (e.g., utilities and materials reduction versus meeting carbon emissions/net zero goals).

Examining the energy landscape, the batch alternative exhibited the highest energy consumption, primarily attributed to CIP cleaning and the electricity demands of large stainless-steel facilities. Amongst the continuous flowsheets, mAb production with protein A capture demonstrated the highest energy efficiency. When precipitation was chosen for the capture step, higher energy demands, particularly due to the large reagents preparation and consumables emissions, were expected comparing to protein A capture. Optimisation efforts, including increasing cell culture productivity, demonstrated considerable reductions in PCF across all flowsheets, with the precipitation flowsheet experiencing the most significant reduction. Finally, this chapter provided a broader perspective by translating PCF into day-to-day relatable metrics, such as equivalent emissions from intercontinental flights or the area of trees needed for carbon offsetting. The benchmarks highlighted that, after flowsheets optimisation, 30 to 50 hectares planted with trees were still needed to offset the carbon emissions of an annual production of 500 kg of mAb across flowsheets.

Extending the simulation framework with the LCA methodology presented in this chapter elevated the added value of the decision-support tool, as full

process economics coupled with comprehensive environmental evaluation help to better understanding the potential of mAb production alternatives.

Chapter 5: Economic impact of implementing process analytical technologies (PAT) in end-to-end continuous antibody manufacture

5.1 Introduction

Process analytical technologies (PAT) comprise advanced analytical techniques, sensors, and data-driven strategies that enable real-time monitoring, control and optimisation of critical parameters. In the dynamic landscape of mAb continuous manufacturing, the adoption of these technologies is expected to offer several economic benefits; however, exact cost savings are not yet clear and this information is shown to be critical to help the business case of PAT implementation.

This chapter delves into the acceptance of PAT across the sector and provides insights into the most commonly used technologies in bioprocessing. The current large-scale implementation level and the barriers that might be hindering the widespread application of these tools are also unveiled through the results of an industrial survey.

Several implementation scenarios comprising different levels of investment and cost savings are also shared. These studies aim to provide evidence on the economic trade-offs of PAT and contribute to a better-informed decision-making related to PAT in continuous mAb manufacturing.

Section 5.2 presents the questions included in the industrial survey and the methodology followed, including selected technologies and process changes, when assessing PAT integration from an economic perspective. **Section 5.3** starts by delving into the survey results and shares the economic outputs from bioprocesses with integrated process analytical technologies. **Section 5.4** summarises the main conclusions shared during the chapter.

5.2 Methods

5.2.1 Industrial survey

An industrial survey was designed to gain insight into the impact of process analytical tools on current and future Quality Control & Quality Assurance (QCQA) activities and process understanding in continuous manufacturing. The respondent pool comprised specialists from a wide range of organisations, including contract manufacturers and large biopharmaceutical companies. A mixture of R&D, manufacturing and management members was selected for this study to include broad points of view. The individuals to whom the survey was circulated were asked to either complete the survey or to distribute it to people within their organisations whom they felt were best positioned to provide a response.

The review of PAT technologies presented in **Chapter 1** shed light on the available options and capabilities of these technologies when applied to bioprocesses. From the examples showed, it was clear that PAT could enable several benefits, such as reducing batch failure, improving process performance or reducing costs. However, it was not clear from the literature which of these benefits was the key motivator for the implementation and current PAT interest from bio-industry. Knowing the relative importance of these benefits could help targeting the development of specific techniques and accelerate the level of adoption across the sector. Equally relevant was to understand what might be slowing down PAT application to bioprocesses and discuss the strategies that can help moving PAT faster into continuous facilities.

As the present chapter also aims to provide insights on the economic impact of implementing PAT in continuous mAb manufacture, get benchmarks on the price of these techniques was crucial. At the same time that the process parameters that translate the PAT benefits (e.g., increased batch success rate, increased volumetric productivity, decreased buffer consumption) were changed, the investment on PAT was also added to the equipment costs in the process economics model described in **Chapter 2**.

The simulation tool also computed the period in which cost savings coming from PAT implementation would balance the investment on these technologies; thus,

it was also important to understand the expectations from the respondents regarding payback timelines.

Most of the surveys were followed by online interviews, which enabled further discussion on the topics and explanation of specific efforts from each organisation towards PAT implementation. These interviews also provided an opportunity for respondents to elaborate on their survey responses, share specific examples or case studies from their organisations, and offer insights that may not have been captured by the structured survey questions alone. This qualitative approach complemented the “more quantitative” data gathered through the survey, enriching the overall analysis and findings of the study. **Table 5.1** presents the list of organisations to which the respondents were affiliated and their respective position within the organisation. The individual interviews consisted of 40-minute online meetings and took place on dates between November 2021 and July 2022.

5.2.2 Process Economics with PAT

5.2.2.1 Selection of process analytical technologies

The previous chapters showed that mAb production with protein A chromatography capture was the most economic and environmentally sustainable scheme across the continuous flowsheets studied. Thus, Conti-ProA was selected as the base for the integration of different process analytical technologies. The analysis of the potential of PAT implementation towards cost savings demanded the integration of modified process inputs in the decision-support tool described in **Chapter 2**. The process simulation with new parameters resulted in new COG values that were compared with Conti-ProA’s COG from the base-case flowsheet (as described in **Chapter 3**). **Chapter 1** presented several studies and PAT examples that indicated advantages over uncontrolled processes. However, only a limited number of authors have described PATs with tangible process benefits that could be translated into modified input parameters. These examples were selected, and the improved process parameters were used in the economic model. **Figure 5.1** presents the steps in which the integration of PATs was considered.

Table 5.1 - List of organisations and the position held by the respondents who completed the survey.

Organisation	Position	Interview post survey
3M	Application Engineering Specialist	X
Achilles Therapeutics	Vice-President Bioprocessing	X
Amgen	Vice-President Drug Substance	X
Amgen	Vice-President Process Development	X
AstraZeneca	Executive Director	X
Biogen	Vice-President Global Manufacturing Sciences	X
Biogen	Senior Director	
C&G Therapy Catapult	Industrialisation and Manufacturing Director	X
CPI	Chief Technologist	X
CPI	Principal Strategic Opportunities Manager	X
CSL Behring	Executive Director	X
CSL Behring	Senior Manager Process Development	X
Cytiva	Business Leader	X
Cytiva	Senior Director	X
Eli Lilly	Director Manufacturing	X
Eli Lilly	Director Process Development	X
Evelo Biosciences	Vice-President Bioprocess Development	
FUJIFILM Diosynth	Vice-President Process Development	
GSK	Director	X
GSK	DSP R&D investigator	X
Lonza	Director External Innovation	X
Lund University	Professor	
Merck & Co. (MSD)	Principal Scientist	X
Merck & Co. (MSD)	Senior Director	X
Oxford Biomedica	R&D Team Lead	X
Pall (now Cytiva)	Senior Director	
Pharmaron UK	Senior Technical Specialist	X
Sanofi	Head of Purification Development	X

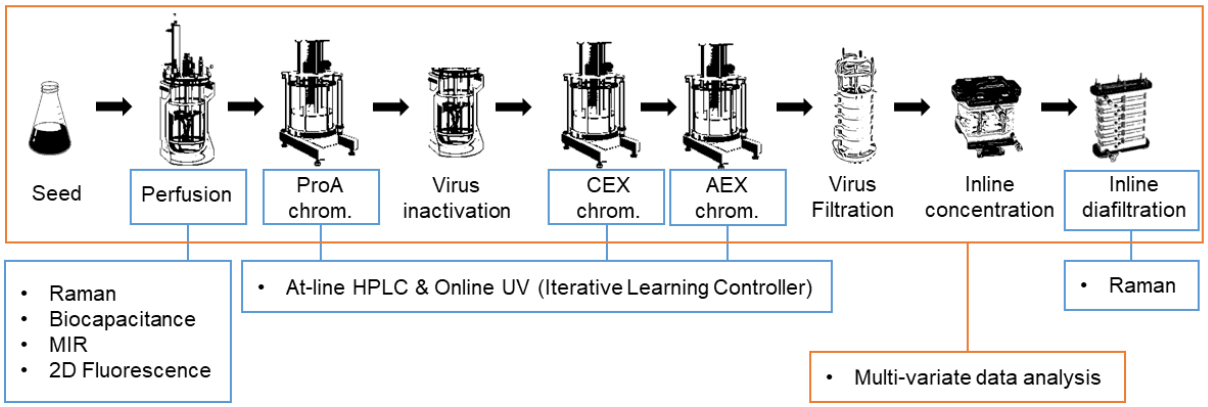


Figure 5.1 - Process flowsheet used in the simulation of PAT integration in mAb manufacture and respective PAT considered. ProA: protein A; CEX: cation exchange; AEX: anion exchange; TFF: tangential flow filtration; SP-TFF: Single-pass tangential flow filtration; MIR: medium infra-red; HPLC: high performance liquid chromatography.

The potential impact of PAT implementation on USP (e.g., volumetric productivity, perfusion rate) and DSP performance (e.g, buffer consumption, resin capacity) were determined through assessing literature reports.

Goldrick et al. (2019) and Esmond-White et al. (2022) utilised multi-variate data analysis (MVDA) via a partial least squares (PLS) model to analyse the data collected in Raman spectra and demonstrated that Raman spectroscopy could significantly impact fermentation or cell culture productivity. Both case studies revealed a 20% increase in output by controlling the concentration of nutrients in solution (e.g., phenylacetic acid for penicillin production, glucose for mAbs) and adjusting the feed rate in the bioreactor. These investigations were conducted in fed-batch mode; however, personal communication with Goldrick's research group confirmed that the level of impact of this PAT would also be expected in continuous mAb manufacture. In the process economics model, the volumetric productivity assumed for Conti-ProA's base-case, 3 g/L/day, was increased to 3.6 g/L/day with the implementation of PAT, to reflect this 20% increase.

With regards to batch success rate, Goldrick et. al (2019) also showed that the control strategy with Raman spectroscopy reduced the number of below-target batches to zero, resulting in an increase from 94% to 100% batch success rate.

A study carried out by Konakovsky et al. (2015) illustrated that biocapacitance probes could be used to achieve real-time biomass control in cell culture, which could also translate into a 2% increase in batch success rate relative to uncontrolled scenarios. Based on personal communication with Goldrick's group, the increase in batch success rate found in batch mode could also be applied in continuous mode. Therefore, the batch success rate in Conti-ProA was increased from 96% to 99%, to reflect an improvement in the range of 2% (Konakovsky et al., 2015) to 6% (Goldrick et al., 2019) by implementing PAT.

Moving on to perfusion rate, Ozturk et. al (1996), Moore (2019) and Brunner (2019) used Raman spectroscopy, biocapacitance, and 2D-fluorescence and medium infra-red (MIR), respectively, to monitor media components during cell culture (e.g., glucose and lactate) and control the media quality and feeding regimes, leading to savings in media consumption (Ozturk et al., 1997; Brunner et al., 2019; Moore, Sanford and Zhang, 2019). In Conti-ProA's base-case, the perfusion rate was set at 1.5 vv/day. With optimised media recipes and a better understanding of the cells' needs, a lower rate of fresh media and product removal could be applied, without compromising optimal cell growth conditions. In the PAT-controlled scenario, media savings were translated into a decrease of 20% in perfusion rate (1.2 vv/day).

For DSP, Lofgren et al. (2021) introduced an integrated continuous process with real-time control utilising an at-line HPLC and an online UV monitor to track product concentration in the chromatography loading and eluate streams (Löfgren et al., 2021). This iterative learning controller (ItLC) detected disturbances of product concentration and automatically adjusted the peaks cutoff during elution. Compared to the uncontrolled scheme, the authors reported a ~25% increase in resin capacity (associated to more product being purified in each cycle compared to the process without the controller) and a 20% decrease in buffer consumption. In the process economics model, these benefits were reflected by changing the binding capacity of ProA from 65 to 74 g mAb/ml resin and CEX and AEX resins from 100 to 124 g mAb/ml resin. For simplicity, the decrease in buffer consumption was simulated by assuming 80% of the base-case column-volumes (CVs) in every chromatography step.

With regards to membrane lifespan, Raman spectroscopy was also employed during DSP by Virtanen et al. (2017), who detected early-stage membrane fouling by monitoring product concentration in the concentrated stream during ultrafiltration (Virtanen et al., 2017). Principal component analysis (PCA) analysed the variation within the Raman spectra and accurately identified the moment when membranes should be replaced during the process. Utilising this PAT demonstrated that membrane lifespan (i.e., operational time before switching) could be extended if fouling was monitored and assuming that there would be no concerns from a regulatory perspective. ILC membranes in Conti-ProA operate for 4 days before replacement, meaning that, per 20 days continuous cycle, 5 sets are required. Personal communication with a leading membrane vendor (Gregor Kalinowski, Pall Life Sciences (now Cytiva), Germany) supported the assumption of extending operation time from 4 to 5 days (25%) with PAT control, decreasing the total number of filters from 5 to 4 per continuous cycle. This increase in membrane lifespan has also an impact on the number of QCQA tests that changed from 5 to 4 per continuous cycle.

Some of previous examples already showcased the benefits of utilising multivariate data analysis coupled with PAT sensors to monitor and control USP and DSP. Other direct cost benefits were discussed during the interviews in the industrial survey. Respondents anticipated that a full integrated continuous process with PAT and MVDA could result in savings of approximately 20% in QC materials and labour costs. For simplicity, this was translated into the economic model by reducing the QCQA test cost from 35 to 28 k$ per batch release and directly reducing QC labour (calculated in **Chapter 2** through the equations shown in **Table 2.2**) by 20%. Additionally, the number of operators required in the plant could be significantly reduced with the increased automation and fewer in-process sampling. Schmidt et al. (2021) estimated a 50% decrease in labour by implementing a digital-twin based process in the continuous production of an mRNA vaccine for SARS-COVID-19. Discussions with industry experts revealed that similar reductions could be expected in continuous mAb manufacture. To reflect this PAT benefit, the number of operators in the process economics model was reduced from 6 to 3 (1 USP, 1 DSP, 1 checker) per shift.

These last PAT benefits (decrease in QC materials and labour costs and the number of USP and DSP operators) were only simulated when all other USP and DSP PAT strategies were also implemented, reflecting the full PAT integrated scenario.

Table 5.2 summarises the changes in input parameters in the process economics model.

Table 5.2 – Process changes simulated in Conti-ProA for the integration of PAT per production area.

Area of PAT integration	Improved parameter	Change to base-case
USP	Perfusion volumetric productivity	+20%
	Perfusion rate	-20%
	Batch success rate	+3%
DSP	Buffer consumption	-20%
	Resin capacity	+25%
	Days of membrane lifespan	+25%
USP+DSP (incl. MVDA)	(all the above)	(all the above)
	QC materials cost	-20%
	QC labour costs	-20%
	Number of operators	-50%

5.2.2.2 Investment on process analytical technologies

The implementation of PAT strategies entails investing in analytical technologies, which must be factored into the total equipment costs in the process economics model.

Table 5.3 shows the prices obtained from research projects within UCL Decisional Tools group and through personal communication with companies that have already invested in these tools (data from survey).

Table 5.3 – Equipment costs assumed in the integration of PAT in Conti-ProA

PAT	Price	Process step & benefits
Raman (device + 4 probes)	400 k$	Cell culture: increased volumetric productivity; increased batch success rate; decreased perfusion rate ILC: increased days of lifespan
Biocapacitance	200 k$	Cell culture: increased batch success rate; decreased perfusion rate
At-line HPLC (2 systems)	200 k$	ProA chromatography, CEX and AEX: increased resin capacity; decreased buffer consumption.

As mentioned earlier, several technologies could yield similar PAT benefits; thus, it was assumed that the PAT investment would correspond to the most expensive option per process step. The investment in PAT in USP was 400 k$, allocated to Raman spectroscopy, while in DSP it was 600 k$, which included 2 HPLC apparatus from the iterative learning controller (personal communication, Bernt Nilsson, Lund University, Sweden) and the device for Raman spectroscopy. The scenario reflecting PAT integration across the entire process was simulated with an investment of 1 M$ (Raman in USP, Raman in DSP and the iterative learning controller in DSP). The investment in MVDA or model validation was not incorporated into the COG model, as it was assumed to be part of the prior process development phase. Additionally, based on the survey responses regarding the impact of PAT on the total consumables and reagents costs of mAb manufacture, these were considered negligible and not added to the simulation.

5.2.2.3 Target analysis

The economic evaluation of the impact of PAT implementation included a target analysis based on the level of PAT benefits and investment. Three levels of implementation were explored: low, medium and high. The process

improvements and technology investment presented in **Table 5.2** and **Table 5.3**, respectively, were considered as the medium level. The low and higher levels were designed with the aim of understanding if the cost savings would significantly if the PAT benefits were more modest or more accentuated than expected. The changes captured at each level of PAT implementation are presented in **Table 5.4.**

Table 5.4 - Process changes simulated in Conti-ProA to reflect different levels of implementation. The batch success rate in the high impact was assumed as 100%. The change in increased days of membrane lifespan was of 0.5 days from the medium (5 days) to low (4.5 days) and high (5.5 days). The number of QCQA tests based on the membrane lifespan was kept at 4/batch for low, medium and high impact. The number of operators was 4 in the low impact and 2 in the high.

	Low	**Medium**	**High**
Benefits			
Perfusion volumetric productivity	+10%	+20%	+30%
Perfusion rate	-10%	-20%	-30%
Batch success rate	+1%	+3%	+4%
Buffer consumption	-10%	-20%	-30%
Resin capacity	+12%	+24%	+30%
Days of membrane lifespan	+15%	+25%	+35%
QC materials cost	-10%	-20%	-30%
QC labour costs	-10%	-20%	-30%
Number of operators	-33%	-50%	-67%
Investment			
Raman spectroscopy	200 k$	400 k$	600 k$
Iterative learning controller	100 k$	200 k$	400 k$
Integrated (2 Raman + ItLC)	500 k$	1 M$	1600 k$

After simulating the cost savings for each scenario, the number of batches required to payback the investment in PAT was calculated according to equation 5.1. The number of batches was used instead the number of years, as it was observed that for the majority of the scenarios the return would be considerably fast (<1 year). As described in **Chapter 2**, for Conti-ProA, the process economics model simulated 10 batches per year.

$$N_{batches} = \frac{\text{PAT Investment}}{(COG_{Traditional} - COG_{PAT}) \times \frac{Demand}{Annual\ batches}} \quad (5.1)$$

Where $N_{batches}$: Number of batches required so the cost savings from PAT implementation would balance the investment

$PAT\ Investment$: Investment in specific PAT ($)

$COG_{Traditional}$: Cost of goods computed for the scenario using Conti-ProA without PAT, as the base-case from **Chapter 3** ($/g)

COG_{PAT} : Cost of goods computed for the scenario using Conti-ProA flowsheet with PAT ($/g)

$Demand$: Annual product demand (g/year)

$Annual\ batches$: Number of batches per year (batches/year)

5.3 Results and discussion

This section presents an analysis of the PAT survey results followed by an economic analysis of the impact of PAT implementation on the process economics of continuous bioprocessing. More specifically, the views of the biopharmaceutical sector on the current state of play and future potential of PAT implementation are discussed; these were solicited via an international industrial survey combined with follow-on one-to-one interviews with industrialists. The survey results fed into an evaluation of the potential economic benefits of PAT. The COG savings resulting from different PAT benefits compared to uncontrolled processes are presented. This analysis was extended by understanding the cost categories (e.g., reagents costs, QC costs, indirect costs) that would benefit the most from each PAT implementation strategy. The last stage of the assessment highlighted the expected number of batches required for the cost savings to balance the investment in enhanced analytics and control.

5.3.1 Industrial survey

The results of the survey are based on the responses of 28 experts. From these 28 respondents, 25 provided further insights via an online interview. The next sections will provide insights on PAT viewpoints and trends within the sector.

5.3.1.1 *"What is the key motivation for PAT implementation?"*

Respondents were asked to rate the following potential PAT benefits in terms of their relative importance: reduced batch failure, increased process performance and reduced QCQA and labour costs. **Figure 5.2** presents the distribution of responses per PAT benefit.

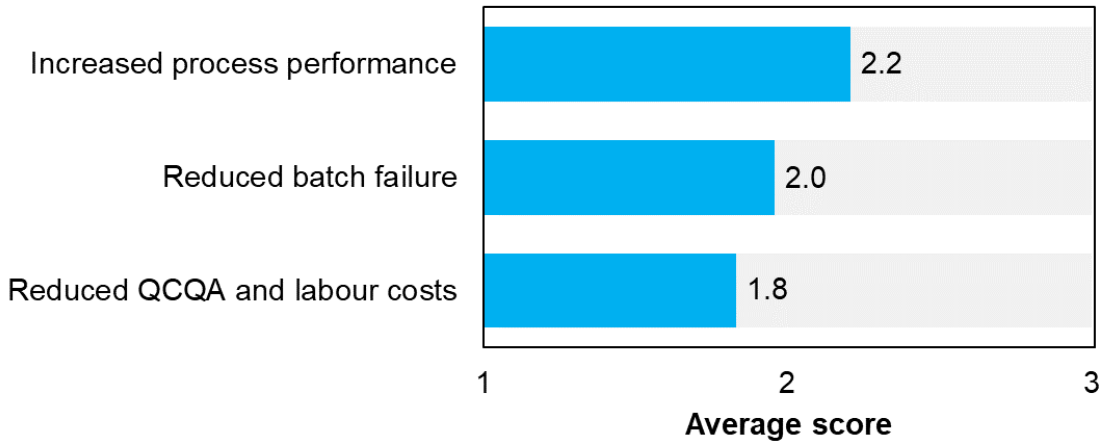


Figure 5.2 – Score reflecting the importance of different benefits when considering PAT implementation in mAb manufacture. The survey results were converted to a score where 3 represented a high level of importance and 1 representing a low level of importance.

Figure 5.2 highlights that the benefit of increasing process performance was slightly favoured over reducing batch failure or reducing QCQA and labour costs; however, all benefits showed a similar level of importance. The interviews shed further light on the reasons for prioritising a particular benefit that were found to be highly company-specific.

Participants in the pool of companies that gave "increased process performance" the highest importance suggested that the benefits from process intensification attained from continuous biomanufacturing could be augmented through the implementation of PAT. In general, companies which envision improved processes through PAT implementation also implemented PAT in a process development (PD) stage to help optimising their commercial scale. In PD, GSK is currently using automated samplers and SEC methods in the chromatography steps with the goal of monitoring the process and maximising yields and purity. Additionally, Cytiva shared their work with AstraZeneca on using PAT and MVDA to unlock benefits related to early column aging and fouling detection, also in chromatography (Ravi et al., 2023). In the upstream process development, AstraZeneca and Eli Lilly have investigated the use of Raman spectroscopy to monitor nutrients' concentration and optimise cell culture outputs (Goldrick et al., 2020; Yousefi-Darani et al., 2022). In contrast, there were respondents who believed that mAb processes were currently

relatively well designed and optimised and that the installation of PAT would not lead to significant increases in process performance. These respondents belonged to the pool of companies whose PAT implementation efforts were driven by the benefits of reducing batch failure.

Several respondents emphasised the importance of reducing batch failure and how PAT could be beneficial in reducing process variability and improving control. Even if current batch failure rates for mAb manufacturing are relatively low (1 to 5%), each batch loss represents a significant impact on a company's cash flow (e.g. $1-8M per batch depending on the production scale (based on Chapter 3 for 100 to 1000 kg/year)). Many participants highlighted that the importance of PAT to monitor in-process parameters in continuous manufacturing may be even higher than in batch, due to the (almost) uninterrupted stream of product from one unit operation to the other (ideally, without holding times) and potentially a higher reliance on final batch release testing. Merck, for instance, confirmed its keenness on implementing real-time in-process testing and shared results from their already published work on using online liquid chromatography (LC) to monitor impurities and avoid out-of-specification batches (Patel et al, 2017). Additionally, there were companies emphasising that the time that they are currently spending on offline testing is slowing down crucial stages of decision-making and can be the cause of sacrificing partial or entire batches. In general, companies that were focused on improving their batch success rates were developing PATs and integrating MVDA techniques with the aim of creating robust inline feedback controls. Many respondents relayed their efforts transitioning from offline testing to using real-time or near-real-time data obtained from inline and at-line PATs to monitor mAb production. For this, critical process parameters (CPPs) such as temperature, pressure, flow rates, or pH, and critical quality attributes (CQAs), such as purity, are continuously analysed and compared to established models so the process conditions are automatically adjusted during manufacture.

With a small margin of difference, QCQA and labour cost savings were the third ranked PAT benefit amongst the surveyed experts. Many of the respondents acknowledged the importance of reducing offline in-process and drug substance testing in mAb manufacturing. Participants shared that 20 to 50%

QCQA and labour savings would be expected after PAT implementation in continuous mAb manufacture. This information fed directly into this thesis' assumptions for PAT economic evaluation. In general, companies that were focused on decreasing QCQA and labour costs were also developing multivariate data analysis models coupled with inline testing. These models can interpret complex data and establish relationships between multiple variables; therefore, decreasing the number of tests (and sampling) needed during the process. From a regulatory point of view, it was interesting to witness that different opinions rule the sector concerning implementing inline testing. Whilst there were companies that suggested that regulatory agencies would easily follow the efforts on switching from offline towards inline testing, there were others that found it more challenging, as the validation of new inline testing can be complex and time-consuming.

When enquired about other key motivators for the implementation of PAT in mAb manufacture, the most prevalent additional factor suggested by the respondents was reducing the waiting times for product release. Regular batch release takes, approximately, 45 to 60 days (reference given by CSL Behring and Eli Lilly), and, as an example, with PAT implementation Eli Lilly is on its way to reducing this time to 30 days or lower. According to the interviewed experts, a faster release of material would bring significant advantages in terms of flexibility, planning and storage. The faster in-process testing could also allow for faster cycles and increasing the annual productivities. Interestingly, there were companies that viewed PAT implementation as a move towards increased modernisation and innovation and were keen to act as trendsetters for the sector.

5.3.1.2 "Where has PAT been implemented?"

Turning the attention to the level of PAT adoption in biopharma, the survey aimed to understand the process steps in which PAT has been integrated across the sector. (**Figure 5.3**).

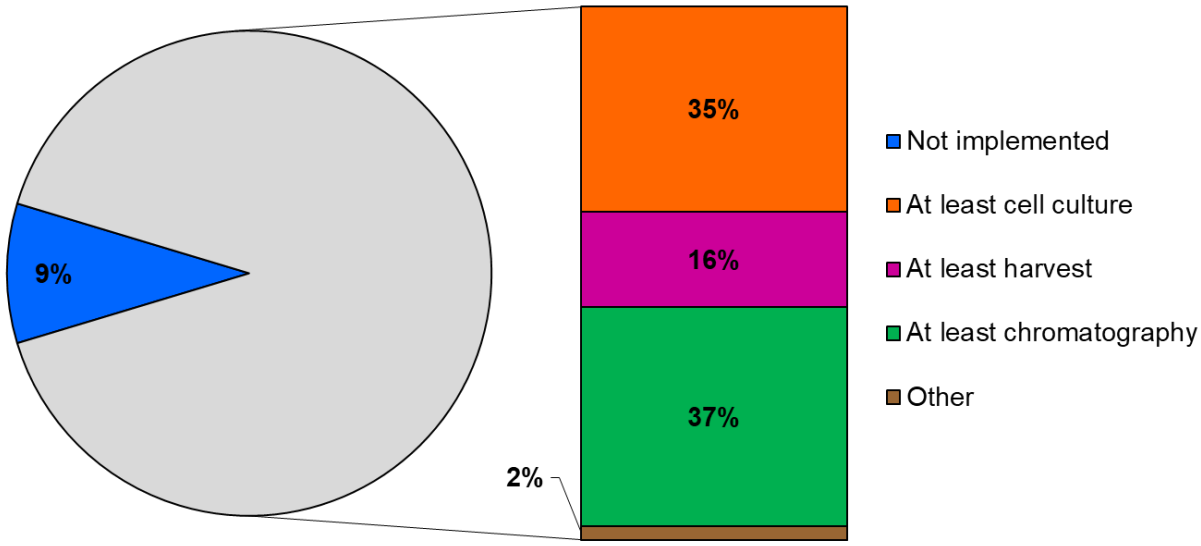


Figure 5.3 – Distribution of PAT implementation per production step across the interviewed companies. “Other” refers to PAT installed for sample automation.

The results showed that more than 90% of the respondents were from companies that, by 2022, had already adopted some form of process analytical technology either in process development or manufacture. Cell culture and chromatography were the process steps with the largest implementation rate. Moreover, from the interviews it was possible to uncover the clear link between the level of PAT implementation and companies with ongoing efforts towards switching to end-to-end continuous processes. Raman spectroscopy was the technology with the highest adoption rate. Published work from the interviewed companies on Raman spectroscopy include Goldrick et al. (2020), Eyster et al. (2021) and Darani et al. (2022). Sanofi disclosed the development of UV-based column switching in continuous capture (Godawat et al., 2012), and Evelo Biosciences shared the usage of near-real-time UPLC coupled with models, also for chromatography monitoring. As aforementioned, Merck had also developed online LC for the monitoring of impurities in mAb manufacture. Other adopted technologies included biocapacitance in cell culture, UV-based loading and eluting, online LC and online SEC-UPLC in chromatography. From the pool of respondents who had not implemented PAT (9%), most of them shared their intention to invest in these technologies to realise the benefits related to a better-controlled manufacture.

5.3.1.3 “What is slowing down the implementation of PAT in mAb manufacture?”

Moving on to the factors slowing down the implementation of PAT, the respondents raised the following issues:

- Technology readiness level (TRL);
- Difficult implementation;
- Sector mind-set;
- Regulatory uncertainties; and
- Lack of knowledge regarding concrete economic benefits.

Regarding the TRL and implementation challenges, many respondents highlighted that, as the development of PATs is very product and process specific, there is a lack of robustness, reliability, or scalability still required for widespread adoption. Additionally, the implementation of PAT often requires that the companies possess expertise on data processing and integration, which may be limiting. Linking to the major QCQA bottlenecks, it was also suggested that, for companies looking for improving quality control, advanced analytical options to detect bioburden, for instance, needed further maturation to justify the investment in these technologies. Respondents also pointed out the challenge associated with the pre-treatment of samples required in many analytical tests. The implementation of inline small buffer exchange steps prior to inline testing may also add complexity to these technologies’ implementation.

Transitioning to the mind-set, implementing PAT often requires organisational shifts towards changing, which can encounter resistance and inertia, especially for well-stablished production platforms, such as mAb manufacture. The participants expressed that this is intrinsically related to the first two mentioned topics, as new technologies or complex data-driven methods can be the source of reluctance within the sector.

The hindering factor of regulatory uncertainty was frequently seen in the survey responses as well. As aforementioned, some respondents shared their opinion that it can be challenging to move to inline testing. While regulatory agencies have made strides in recognising the potential benefits of PAT, there is still variability in their acceptance and validation of PAT technologies. These concerns were echoed by respondents who had to undergo intensive validation to replace offline with inline testing. Companies must demonstrate that inline testing does not compromise product safety, efficacy, or regulatory compliance and that appropriate controls are in place to mitigate risks associated with PAT implementation (e.g., unexpected process variability and modelling variation). On the other hand, there were also participants who suggested that by fostering open communication and transparency about their efforts and protocols, the sector could build credibility with regulatory reviewers and navigate the regulatory approval process more effectively.

Regarding the lack of information on economic benefits, participants shared that, although cost savings are expected, many companies are waiting for evidence of a clear return on investment from those that have already implemented PAT. As discussed in **Section 5.2**, although there were many examples of improvements through PAT implementation, reports on tangible cost reductions were limited. In this thesis, an economic evaluation encompassing the analysis of COG savings for different implementation strategies will be shared. This will provide extra insight to the sector and, hopefully, accelerate the decision-making about PAT implementation.

5.3.1.4 "What is the expected timeline for widespread PAT implementation?"

The final stage of the survey explored the view of biopharma towards the future of PAT implementation, including timelines and economic insights. **Figure 5.4** presents respondents' selection of the timeframes expected for the widespread integration of PAT in continuous bioprocesses.

The results revealed an optimistic short-term outlook, as a combined total of 41% of respondents anticipated seeing widespread PAT implementation within

3 years. Interestingly, another 33% of respondents foresaw implementation within 4 to 5 years, which means that the vast majority of the survey participants (74%) anticipated the materialisation of continuous bioprocesses with PAT in less than 5 years. These results supported the findings from **Figure 5.3**, where 90% of the interviewed companies had already shared the application of PAT to their processes.

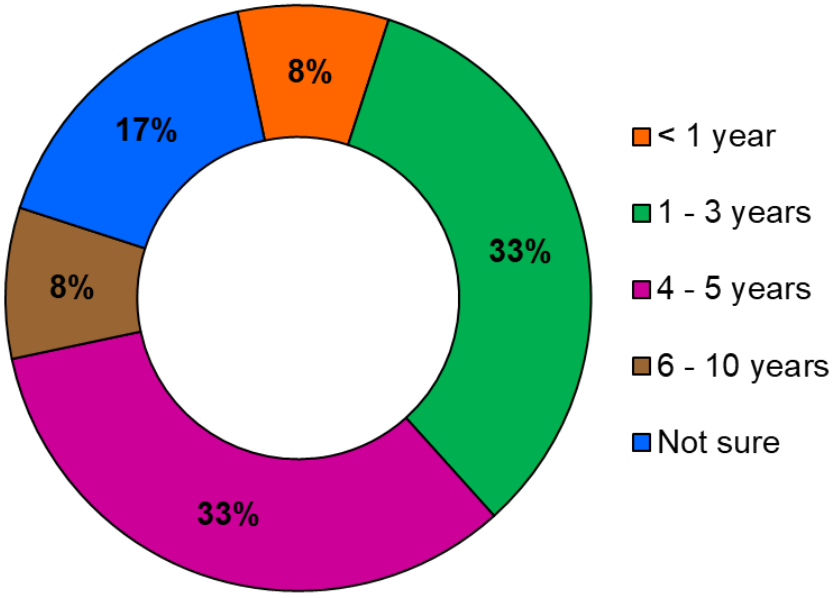


Figure 5.4 – Expected timeline for widespread PAT implementation across the biopharmaceutical sector.

5.3.1.5 "What is the desired payback time for PAT investment?"

Prior to the economic evaluation presented in the next section, it was also important to understand the respondents' time expectations for PAT financial returns. The selected timeframes showed in **Figure 5.5** corresponded to the periods after which the respondents would desire that cost savings coming from PAT implementation would balance their investment on these technologies.

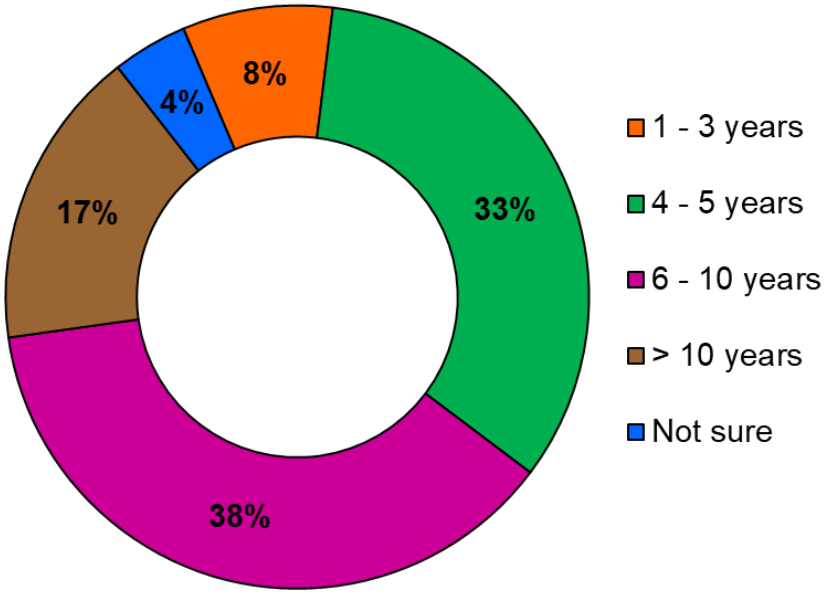


Figure 5.5 – Desired payback times (years) for PAT investment.

According to **Figure 5.5**, the majority of the respondents (38%) would consider to investing in PAT even if cost savings would be seen only after 6 to 10 years. Also, there was a considerable percentage of participants (17%) that would consider to investing if the economic returns would take more than 10 years, emphasising that the pursuit of technology modernisation was more relevant than rapid profitability. Interestingly, most of the respondents who selected the ranges "1 - 3 years" or "4 - 5 years" expressed their beliefs that cost savings from PAT would enable shorter payback times. Therefore, longer timeframes, such as more than 5 years, would be out of scope.

5.3.2 Cost-benefit and environmental analysis of PAT implementation in continuous processes

The economic and environmental consequences of implementing PAT in continuous processes were assessed using the decisional tool introduced in **Chapter 2**. This section starts by providing insights on the economic and environmental outputs from controlled flowsheets compared to traditional uncontrolled schemes. The analysis is then extended by offering a target

analysis showing the impact of PAT on COG savings and technology payback time at different conditions.

5.3.2.1 Cost analysis of PAT implementation

A detailed economic assessment of PAT implementation was carried out, examining the trade-offs between different PAT instruments and their adoption across different process stages, specifically USP versus DSP. Raman spectroscopy and an iterative learning controller (ItLC) were the technologies selected for this analysis due to their broad applicability and potential benefits in USP and/or DSP.

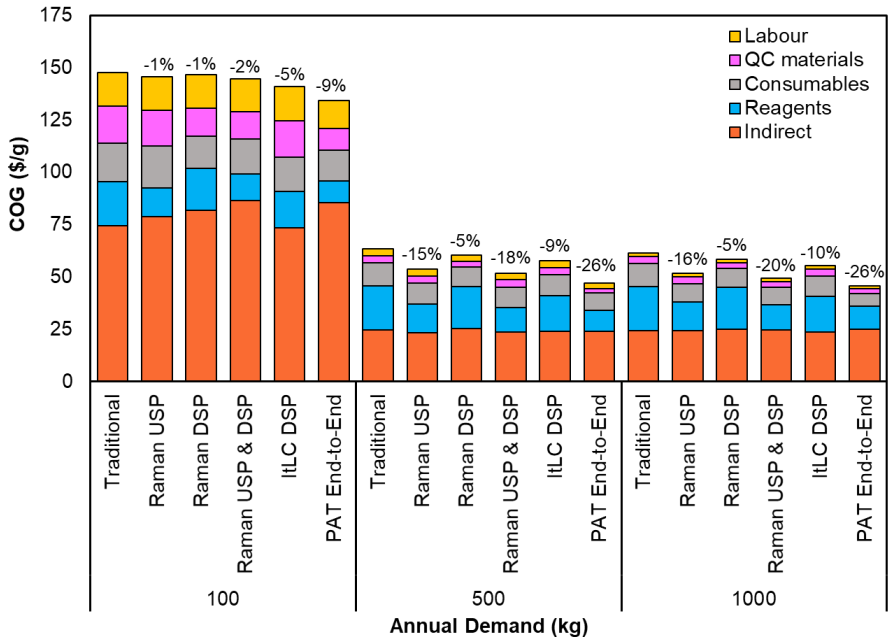
Figure 5.6 outlines the economic impact of different PAT instruments across different productions scales in terms of COG/g for the traditional uncontrolled Conti-ProA and controlled flowsheets adopting one or more PAT instruments. When considering which PAT technology (Raman vs. ItLC) to invest in, **Figure 5.6** highlights that Raman offers greater cost efficiency at medium and larger scales, while the installation of either Raman spectroscopy or ItLC did not demonstrate major benefits at the lower 100 kg/year scale (-1% to -4% across single implementation scenarios). At both 500 and 1000 kg/year, Raman spectroscopy showed COG savings of ~20% and ~15% when installed in USP and DSP or in USP only, respectively. Raman at DSP only did not show relevant cost benefits. At the same scales, the iterative learning controller showed a ~10% cost reduction.

When considering which stage to focus on for PAT implementation (USP vs. DSP), Raman spectroscopy and the ItLC were combined in the DSP flowsheet and compared to the scenario with Raman in USP (now “PAT USP”). **Figure 5.7** illustrates once more the small benefits from PAT at low scale and shows that focusing on PAT implementation in USP is more advantageous than implementation in DSP at medium and larger scales.

The increased COG savings from lower to medium and higher scales was explained by the fixed PAT investment being spread over a larger product output and the savings in variable costs (e.g., reagents, consumables) coming from multiple process improvements having a higher impact. The larger benefits

of PAT in USP (i.e., Raman spectroscopy) over the other strategies can be attributed to the significant decrease in reagents costs when installing Raman spectroscopy in cell culture (decrease in media consumption due to higher perfusion volumetric productivity and lower perfusion rate). For PAT in DSP only, the higher indirect costs (due to the investment in a 400k$ Raman system and a 200k$ ItLC) and higher reagents costs relative to PAT in USP outweighed the savings in consumables and QCQA costs.

In the previous section, the results from the survey explored the opinion of the respondents on the benefits on the QCQA and labour savings of adopting PAT and the associated MVDA across the end-to-end process. The PAT End-to-End flowsheet represented in **Figure 5.6** and **Figure 5.7** reflected the additional advantages from reducing the QCQA materials and labour costs and the number of operators relative to Raman and ItLC process improvements only and included PAT implemented in both USP and DSP. This flowsheet was the one presenting the highest COG savings across scales (COG savings from PAT End-to-End flowsheet: -9% at 100 kg/year; -26% at 500 kg/year and 1000 kg/year).



↑ Perf. Volum. Productivity (USP)	-	X	-	X	-	X	-	X	-	X	-	X	-	X	-	X	-	X
↓ Perfusion rate (USP)	-	X	-	X	-	X	-	X	-	X	-	X	-	X	-	X	-	X
↑ Batch success rate (USP)	-	X	-	X	-	X	-	X	-	X	-	X	-	X	-	X	-	X
↓ Buffer consumption (DSP)	-	-	-	-	X	X	-	-	-	-	X	X	-	-	-	-	X	X
↑ Resin capacity (DSP)	-	-	-	-	X	X	-	-	-	-	X	X	-	-	-	-	X	X
↑ Membrane lifespan (DSP)	-	-	X	X	-	X	-	-	X	X	-	X	-	-	X	X	-	X
↓ QC materials cost	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-	-	-	X
↓ QC labour cost	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-	-	-	X
↓ Number of operators	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-	-	-	X
PAT investment (M$)	-	0.4	0.4	0.8	0.2	1	-	0.4	0.4	0.8	0.2	1	-	0.8	0.8	1.6	0.4	2
Equipment cost (incl. PAT) (M$)	1.9	2.2	2.3	2.6	2.0	2.6	3.1	2.8	3.5	3.2	3.2	3.6	6.2	5.7	7.0	6.4	6.5	6.7
Bioprocess equipment cost savings (M$)	-	0.1	N/A	0.1	0.1	0.3	-	0.7	N/A	0.7	0.1	0.8	-	1.3	N/A	1.4	0.2	1.5

Figure 5.6 - Breakdown of COG/g on a cost category basis for traditional continuous mAb production and 6 other flowsheets with different PAT technologies (Raman vs. iterative learning controller) at 100, 500 and 1000 kg/year commercial scales. At 1000 kg/year the production platform is built by two identical USP and DSP trains; therefore the equipment investment (including PAT) is approximately the double of the one found for 500 kg/year scenario. The embedded table shows which process benefits are simulated for each flowsheet. The PAT investment is a model input, while the total equipment costs are an output. The equipment cost is the basis for the fixed capital investment (FCI) calculation, from which the indirect costs are derived.

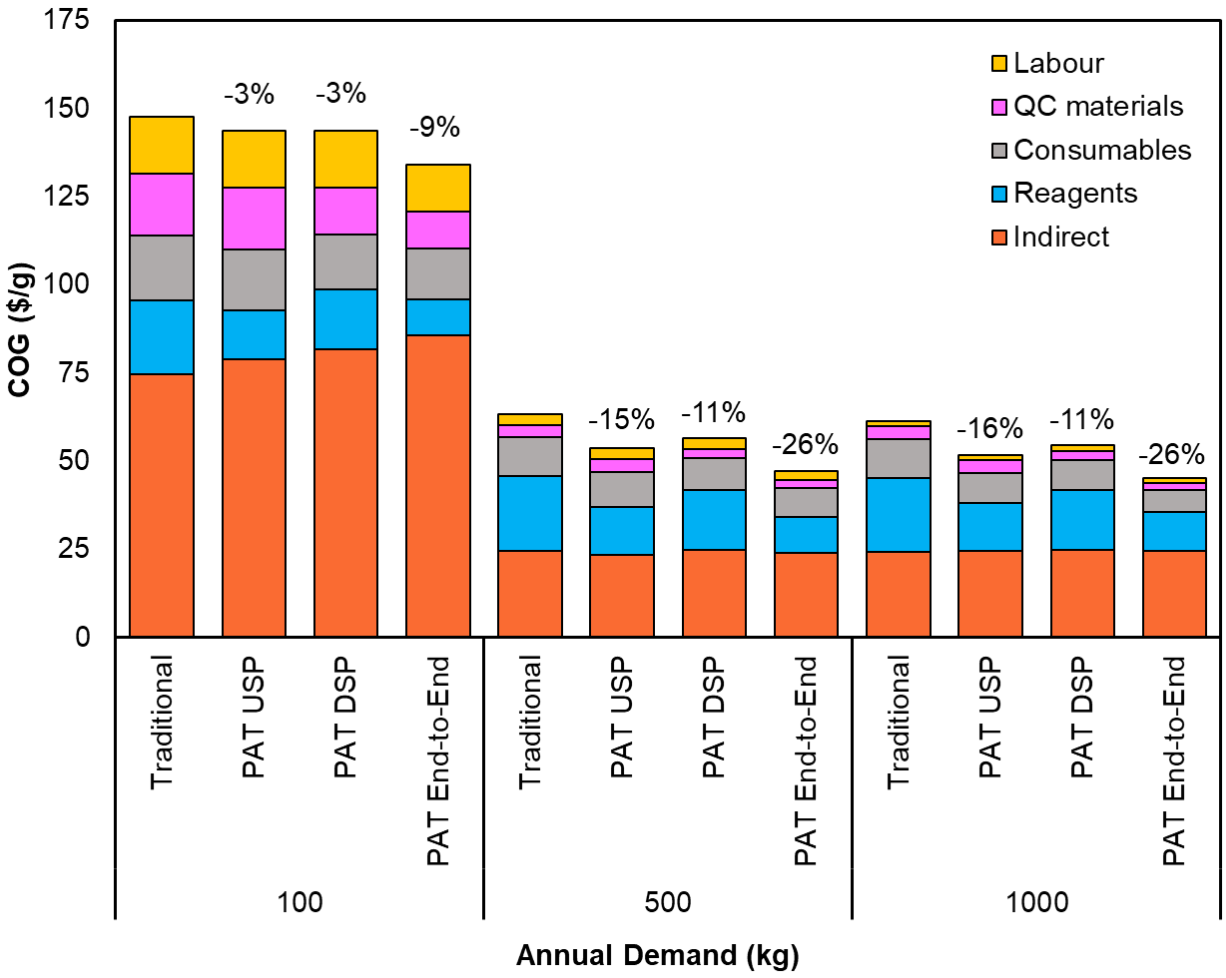


Figure 5.7 - Breakdown of COG/g on a cost category basis for traditional continuous mAb production and three flowsheets with PAT adopted at different stages (USP vs. DSP vs. PAT End-to-End) at 100, 500 and 1000 kg/year commercial scales.

Diving deeper into the nuances of the PAT impact helped in understanding the COG outputs. The radar chart in **Figure 5.8** displays all standardised values for each cost category across each flowsheet and it was used to simplify the visualisation of the PAT impact on each type of cost. Values toward the outer edges of the radar chart indicate relatively higher costs in that category, while values closer to the centre represent relatively lower costs.

In general, the traditional flowsheet demonstrated higher costs across cost categories, with the exception of indirect costs. In contrast, while presenting

high indirect costs, the End-to-End scenario showed the lowest cost outputs in the remainder categories.

Looking at reagents costs, PAT in DSP showed the highest values amongst the controlled flowsheets, as the reduction in chromatography buffer consumption alone was not as substantial as reducing cell culture media (reagents cost savings: -34% PAT in USP; -18% PAT in DSP; -51% PAT End-to-End). In contrast, moving to the consumables costs and QCQA costs, the increase in resin capacity offered by the iterative learning controller and the decrease in number of “membrane replaces/switches” offered by Raman spectroscopy in DSP had a marked effect in the consumables cost. Additionally, the extension in membrane lifespan in PAT DSP through Raman decreased the number of QCQA tests, which had a direct impact on the QCQA materials costs (consumables cost savings: -5 to -10% PAT in USP; -16 to -23% PAT in DSP; -21 to -44% PAT End-to-End; QCQA materials cost savings: 0% PAT in USP; -25% PAT in DSP; -41% PAT End-to-End). The labour costs were similar across flowsheets, with the only significant reduction seen for the End-to-End scenario. For this flowsheet, the number of operators was reduced from 6 to 3, leading to a significant decrease in labour expenses.

The indirect cost category was the only one where the PAT End-to-End scenario showed higher values relative to the other flowsheets. As previously discussed, the additional cost of integrating PAT in mAb manufacture can have an impact on the total equipment purchase cost that influences the final COG. As summarised in the embedded table from **Figure 5.6**, for the PAT End-to-End flowsheet, the investment in PAT equipment is simulated as 1 (for 100 and 500 kg/year) or 2 M$ (for 1000 kg/year, with 2 parallel process trains). At 500 and 1000 kg/year, the decrease in media and buffer volumes with the combined PAT benefits in USP and DSP had a significant impact on reducing the number of purchased SU bag containers and the size of the bioreactor, and this saving exceeded the investment in the PAT.

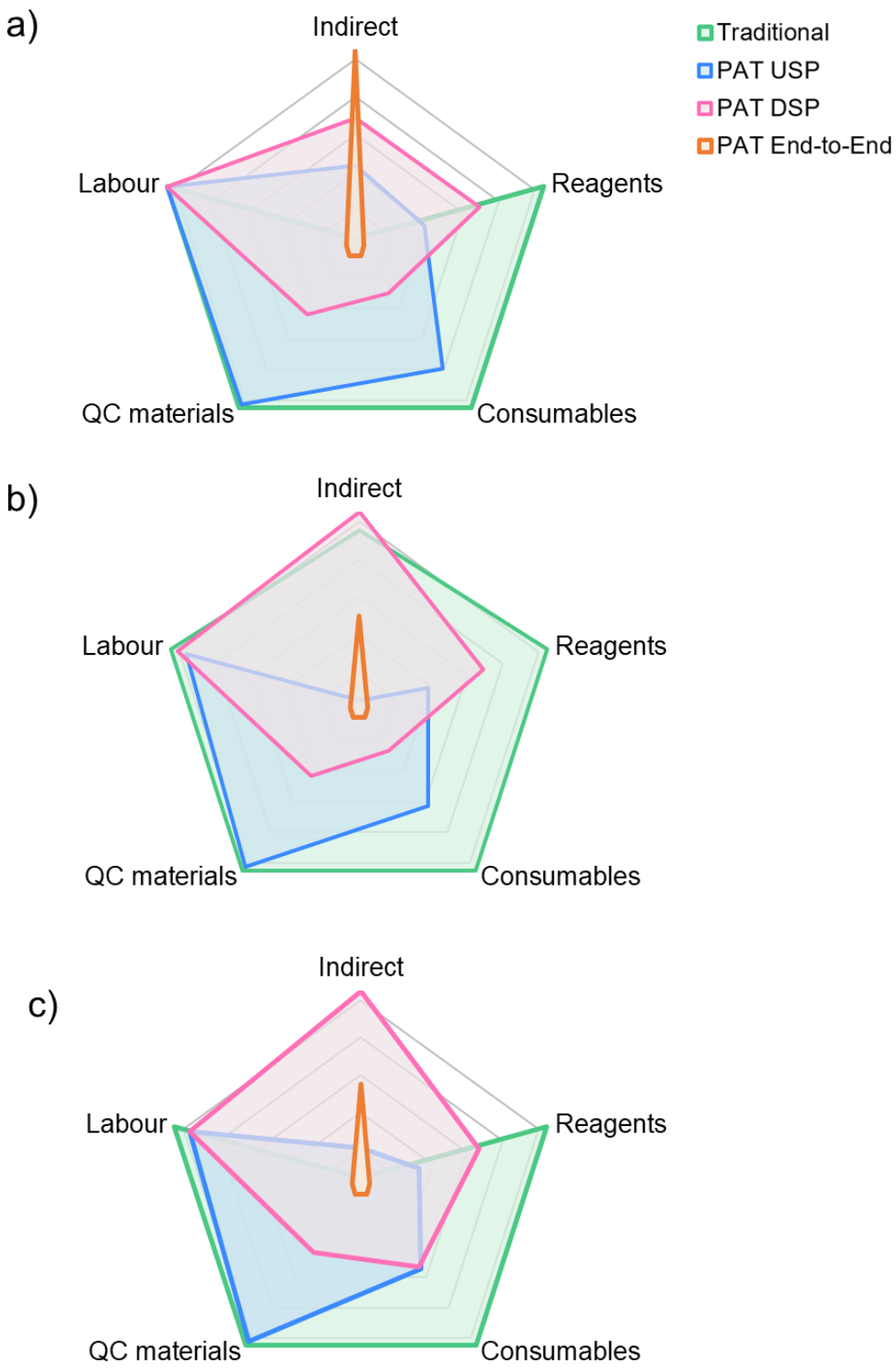


Figure 5.8 - Rating values ($/g converted into 0 to 1 values per category, based on the same methodology as detailed in **Section 3.3.5**) of Traditional Conti-ProA, PAT in USP, PAT in DSP and PAT End-to-End for cost category at a) 100 kg/year, b) 500 kg/year, and c) 1000 kg/year. Higher ratings reflect higher costs and are seen on the outer edges of the chart and represent higher costs.

5.3.2.2 Environmental Analysis

To quantify the broader impact of PAT implementation and the resulting process improvements, the economics analysis was extended with an environmental analysis. The PMI metric captures the reduction in water and consumables waste as a result of the PAT implementation and is a metric that is generated automatically from the cost model. The potential benefit of transitioning from uncontrolled to PAT-based manufacture was evaluated by analysing the PMI of each scenario. The results are shown in **Figure 5.9** and are split into water and consumables PMI for the different production strategies.

The PAT End-to-End flowsheet demonstrated the most substantial reduction in overall PMI compared to the Traditional flowsheet (PMI: 2200 kg/kg Traditional Conti-ProA; 1800 kg/kg PAT USP; 1900 kg/kg PAT DSP; 1500 kg/kg PAT End-to-End across scales). This reduction was driven by a decrease in the water PMI and consumables PMI, coming from the PATs installed in USP and DSP, respectively. The PAT End-to-End flowsheet represented a controlled mAb flowsheet with Raman in USP, Raman in DSP and an iterative learning controller in DSP. The Raman in USP resulted in less media consumption and less SU bags by increasing the perfusion volumetric productivity and decreasing perfusion rate. The iterative learning controller in DSP resulted in lower buffer volumes and less SU bags waste by decreasing buffer consumption, and in lower resin consumption by increasing resin utilisation. Also in DSP, the Raman system resulted in less filters by increasing the membrane lifespan.

Turning to the environmental burden comparison of PAT USP vs. PAT DSP, following the cost analysis where PAT USP generally presented lower COG than PAT DSP, the PMI results also favoured advanced control implementation in upstream processes over downstream.

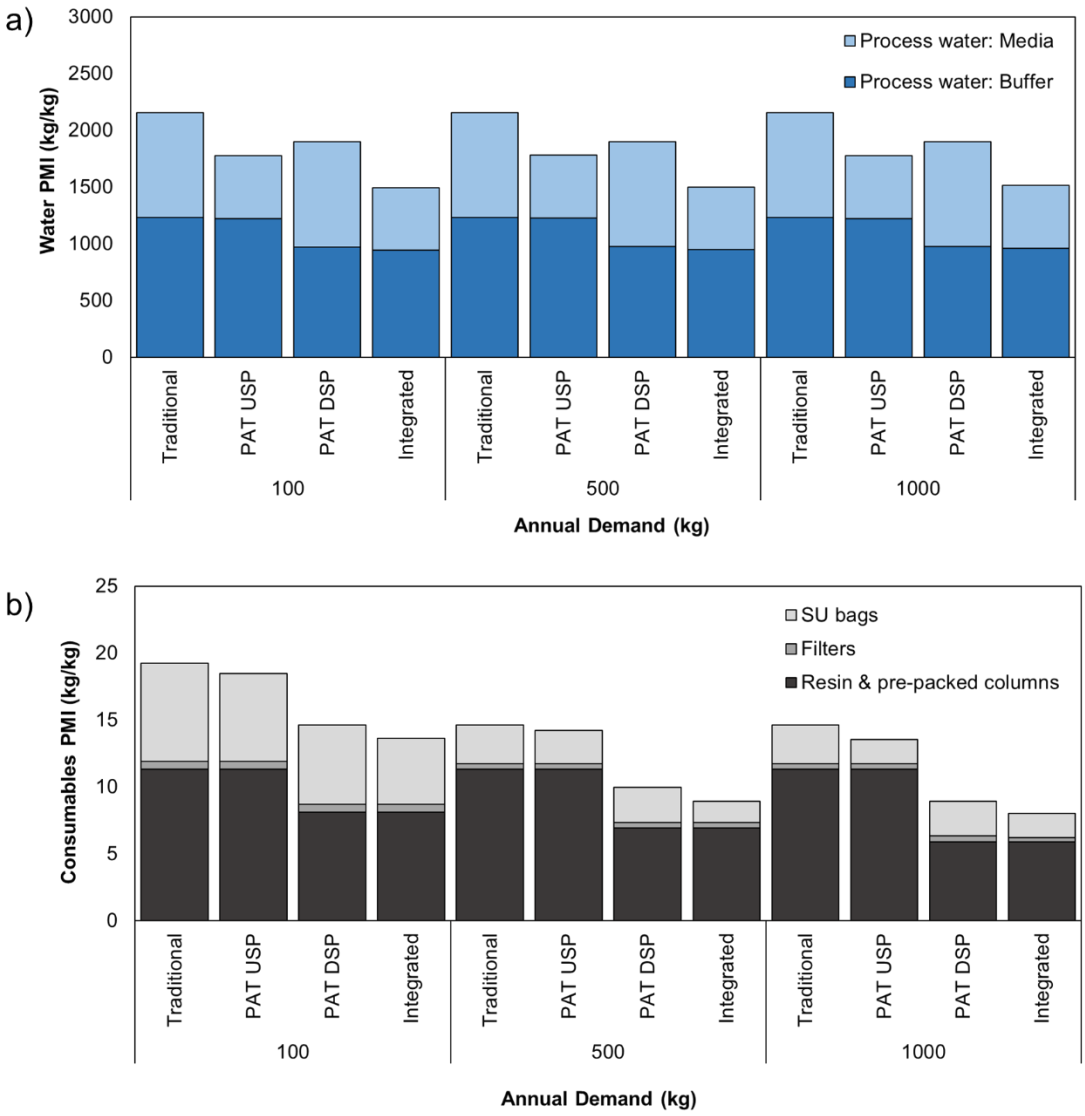


Figure 5.9 - a) Water and b) consumables process mass intensity (PMI) breakdown for Traditional Conti-ProA, PAT in USP, PAT in DSP and PAT End-to-End flowsheets at 100, 500 and 1000 kg/year commercial scales. The water and consumables PMIs include the complete production train liquid and solid waste, respectively. The consumables PMI is based on the total weight of individual disposable material (SU bags, filters, resin and pre-packed columns). The weight of each material was found in literature or given by suppliers. SU bags include both bioreactor bags and buffer hold bags.

As discussed in the cost analysis, the PAT implementation in USP yielded significant savings in media consumption, which were also reflected in a sizable difference in water PMI in PAT USP relative to the Traditional flowsheet.

Conversely, the small savings in chromatography buffers when installing PAT in DSP did not significantly impact water PMI. On the consumables level, the decrease in SU bags from media volume reduction in PAT USP was small compared to the significant decrease in resin and membranes consumption in PAT DSP (e.g. 500 kg/year consumables PMI: 15 kg/kg Traditional Conti-ProA; 14 kg/kg PAT USP; 10 kg/kg PAT DSP; 9 kg/kg PAT End-to-End). As seen in **Chapter 3**, the water PMI (order of thousands of kg/kg) outweighs the consumables PMI (order of tens of kg/kg); therefore, flowsheets leading to smaller water PMIs reflect a lower environmental burden according to this metric.

Overall, the PAT End-to-End flowsheet presented a 30% decrease in overall PMI compared to the Traditional flowsheet, while PAT USP and PAT DSP led to 17% and 12% lower PMIs, respectively. These findings suggest that the environmental benefits of moving from batch to continuous flowsheets with protein A chromatography, as discussed in **Chapters 2** and **3,** could potentially be augmented by integrating process control in these flowsheets.

5.3.2.3 Target Analysis

The COG analysis showed that continuous mAb flowsheets modelled with PAT could offer lower costs compared to the traditional uncontrolled scheme, depending on the scale and stage of implementation. This section begins by highlighting the conditions necessary in terms of process benefits and PAT investment to achieve a target COG saving threshold of at least 20% compared to the continuous flowsheet with ProA capture, thereby justifying the PAT implementation. The process changes implemented and PAT investment assumed were based on **Table 5.4**. This exercise also helped understanding the potential impact of PAT when the investment in such technologies exceeds anticipated values or if the process benefits fail to meet expectations. Error! Reference source not found. summarises the target analysis as a matrix of heatmaps across scales and PAT implementation strategies (PAT in USP vs. PAT in DSP vs. PAT End-to-End PAT). The conditions that met the target 20% COG savings were highlighted by the region within the thick black solid lines.

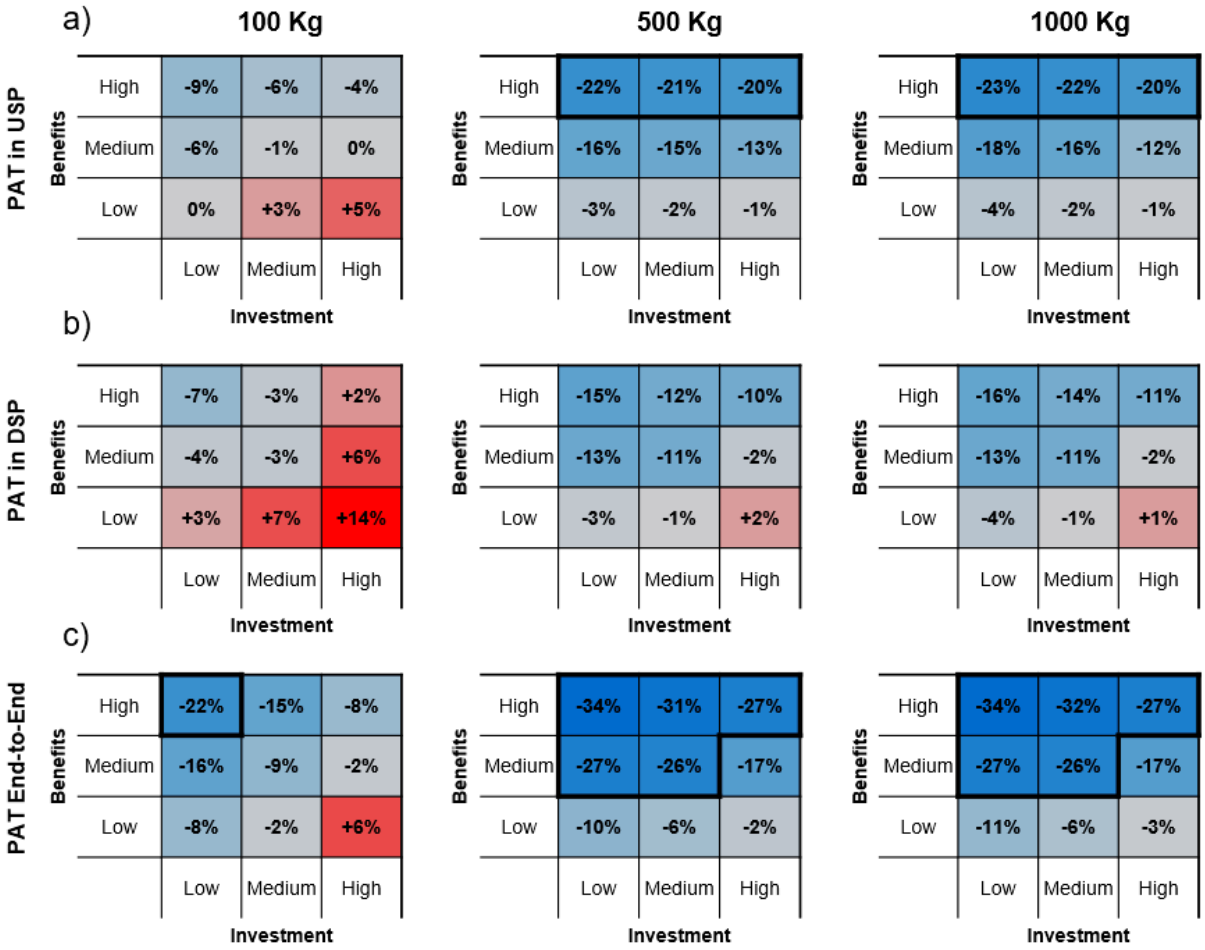


Figure 5.10 - Heat maps showing the COG difference for a) PAT in USP, b) PAT in DSP and c) PAT End-to-End flowsheets relative to Traditional Conti-ProA as a function of the level of process benefit and level of investment. Blue cells represent COG/savings compared to the uncontrolled scheme, while red cells represent higher costs (i.e., when the investment in PAT equipment is not compensated by the associated benefits of implementing PAT). The area within the thick solid black line indicates the conditions at which controlled flowsheets present ≥20% COG/g savings compared to the uncontrolled scheme. The base case is with medium benefits and investment. The levels of investment at 100 and 500 kg/year correspond to 200k$/400k$/600k$ for Low/Medium/High USP; 300k$/600k$/1M$ for Low/Medium/High DSP and 500k$/1M$/1.6M$ for Low/Medium/High PAT End-to-End. The levels of investment at 1000 kg/year correspond to 400k$/800k$/1.2M$ for Low/Medium/High USP; 600k$/1.2M$/2M$ for Low/Medium/High DSP and 1M$/2M$/3.2M$ for Low/Medium/High PAT End-to-End.

Examining **Error! Reference source not found.** vertically (PAT implemented at different stages), the 20% target COG saving was not achieved for flowsheets with PAT in DSP, while PAT in USP and the PAT End-to-End flowsheets showed successful combinations of benefit vs. investment. Given that the ranking of the options was End-to-End >USP>DSP, that trend was also generally mirrored in the size of the window that met the target. Additionally, the 20% target COG saving was not achieved for low levels of process benefits across strategies. While the medium benefit level represented the process improvements gathered from literature or discussed with industrial experts and simulated in the previous cost analysis, the lower benefit levels reflected improvements generally 50% lower than these. This indicated that the investment in PAT would be most attractive when the resulting process improvements are expected to be as significant as those reported in this work.

Looking at the heat maps from left to right (PAT per stage at different scales), it was possible to infer about the impact of production scale in meeting the target cost saving. At 100 kg/year, implementing PAT solely in the upstream (USP) or downstream (DSP) stages did not result in the desired 20% cost reduction. The PAT End-to-End strategy, which covers both USP and DSP, could meet the target only if the investment was halved and/or the process benefits increased by 50%. Additionally, for both USP and DSP-specific PAT implementations, scenarios with low benefits or high investments generally led to processes with higher COG compared to the baseline (as seen by the red-shaded cells). As the production scale increased to 500 kg/year and 1000 kg/year, the range of conditions that could achieve the target cost savings expanded. For example, applying PAT only in the USP could yield savings of ≥20% if the benefits were high, regardless of the investment level. For the PAT End-to-End configuration, cost savings between 25% and 35% were achieved, highlighting the potential of a fully controlled bioprocess as the production scale grows.

To link to the survey results regarding the desired payback time for PAT investment, the number of production batches that would reflect COG savings to offset the investment in enhanced control was calculated. The simulation of Conti-ProA using the process economics model described in **Chapter 2** assumes that each production year consists of 10 batches of 20 days. **Figure**

5.11 illustrates the payback batches, i.e. the number of batches required to pay back the investment in PAT, required for each combination of PAT investment versus benefit.

The solid black lines in **Figure 5.11** highlight the combinations of PAT investment and benefits leading to payback batches below 10 (i.e. 1 year) and reveal that, for the combinations resulting in actual COG savings, the payback was fast for the vast majority of cases (<1 year).

When the decision is based on the payback, the window of combinations that justify the investment is larger than when looking at COG savings only. From top to bottom, PAT in USP and the PAT End-to-End flowsheets showed similar windows. PAT in DSP did not reach the 20% COG savings target for any scale in the previous analysis; however, it could offer payback times shorter than 1 year depending on the conditions.

Looking from left to right, at 100 kg/year, the payback target account be met if the PAT investment and the benefits are low, particularly for the PAT in USP and PAT in DSP scenarios. At 500 and 1000 kg/year, the only combinations that did not meet the target payback were medium to high investment combined with medium to low benefit for PAT in DSP or the “low benefit – high investment” scenario for PAT in USP and End-to-End flowsheets.

The large number of batches seen on the “low/medium benefit – medium/high investment” cells reflects scenarios where PAT implementation is leading to minimal cost savings, therefore, the number of batches needed to meet the investment is accentuated.

Crossing these results with the survey outputs, 8% of the participants indicated that the desirable payback time for PAT investment should be under 3 years, while the remainder pool of respondents suggested that a longer payback would also justify the investment. In the present payback analysis, a large range of PAT investment and resulting benefits were simulated and showed fast payback times for the majority of conditions. Therefore, the findings suggest that PAT in mAb manufacture can potentially satisfy the requirements from industry in terms of payback time.

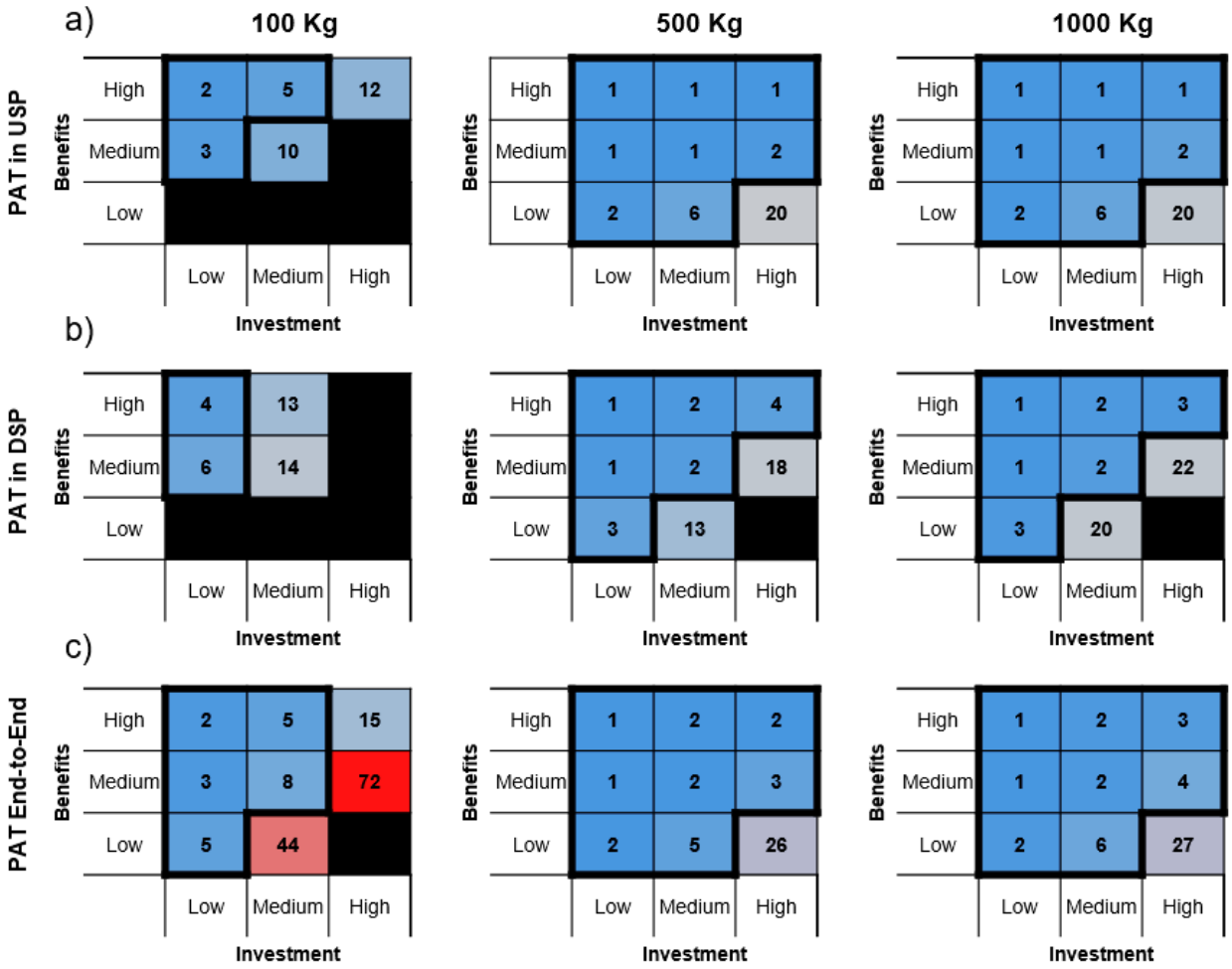


Figure 5.11 - Heat maps showing the payback batches (the number of batches required to payback the investment in PAT) for a) PAT in USP, b) PAT in DSP and c) PAT End-to-End flowsheets relative to Traditional Conti-ProA as a function of the level of process benefit and level of investment. The area within the solid green line indicates the conditions at which the number of batches required is lower than 10 (i.e., 1 production year). The area in black corresponds to combinations that did not result in COG savings. The base case is with medium benefits and investment.

5.4 Conclusions

This chapter evaluated the trade-offs of implementing process analytical technologies in continuous mAb manufacture. The decisional tool was used to assess the implementation of different PAT systems at different process stages from both economic and environmental dimensions. The approach consisted of

configuring PAT process benefits and respective investment into the simulation framework, deriving the cost of goods and process mass intensity metrics and comparing the output from controlled flowsheets with traditional uncontrolled schemes. The analysis demonstrated that end-to-end continuous mAb flowsheets with protein A capture would benefit from the adoption of PAT systems in both USP and DSP. Additionally, the simulations showed that Raman spectroscopy, mainly through the increase in cell culture performance, could have a positive impact on reagents reduction and consequently COG savings. From an environmental perspective, controlled flowsheets showed a smaller PMI, meaning that the environmental benefits from process intensification through continuous manufacture could be augmented with the installation of PAT. The assessment was extended by performing a target analysis showing the level of investment and process improvements required to achieve 20% COG savings relative to end-to-end continuous mAb production without PAT. The analysis showed that a broad range of conditions would meet the target cost reduction at medium and large scales and that savings higher than 30% could be expected. When computing the number of production batches required for the cost of goods savings to balance the investment in PAT, it was clear that the PAT payback period would be fast for an extended array of combinations of investment vs. benefit. A process with PAT implemented end-to-end could deliver payback times shorter than one year for the majority of scenarios tested. Overall, this work helped quantify the expected cost savings from PAT implementation, which can help inform decision-making in the sector regarding the investment in enhanced control.

Chapter 6: Conclusions & future work

The biopharmaceutical industry is pursuing novel strategies to deliver innovative therapies while minimising costs and environmental footprint. Continuous manufacturing is gaining traction as an enabler of smaller facilities and lower resource utilisation, thus acting as a response to the sector's economic and environmental goals. Also, there is a renewed interest in using column-free techniques to decrease the consumption of expensive resins in mAb manufacture. Nevertheless, reports on the evaluation of end-to-end continuous bioprocessing and its benefits are still limited. Also, there is a lack of economic and environmental data to support the decision-making regarding the introduction of column-free alternatives or process analytical tools to decrease costs. In the context of biopharmaceutical decision-making, decisional tools have been used at different stages of the product lifecycle, from early development to commercial production, enabling the analysis of complex alternatives, predict outcomes and optimise various processes. In this thesis, decisional tools were used to explore the economic and environmental trade-offs of batch and continuous mAb manufacturing flowsheets with column-based and column-free capture steps and enhanced control.

This chapter outlines the main conclusions derived from the work developed in this thesis. Additionally, several future developments are suggested to extend the capabilities of the decision-support framework and increase the understanding on continuous mAb manufacture and its potential.

6.1 Overall conclusions

Chapter 3 focused on the detailed application of process modelling to predict cost of goods, capital investment and PMI indicators and derive key cost drivers. The tool to model end-to-end continuous bioprocesses was built on previous UCL work and extended to integrate: i) mass balance and design equations for aqueous-two phase extraction and precipitation; ii) equipment costs and default process parameter values for column-free capture operations and; iii) calculations for environmental metrics with an updated database including masses of consumables.

The results showed that the continuous production strategies, whether ProA-based (Conti-ProA) or column-free, offered COG savings compared to the standard batch (Batch-ProA) at lower and medium scales, while at higher scales only the continuous flowsheets with ProA capture or product precipitation (Conti-PP) presented a similar or slightly lower COG than batch. The cost comparison among continuous flowsheets also showed that the ProA-based flowsheet had the lowest COG across demands, followed closely by product precipitation. The continuous flowsheet with aqueous two-phase extraction as capture step (Conti-ATPE) showed the highest cost of goods amongst all continuous flowsheets. The analysis revealed the underlying cost drivers for each flowsheet, highlighting the significant contribution of large media volumes costs and high equipment costs in the COG of Conti-PP and Conti-ATPE, respectively.

On the environmental front, although the consumables PMI was 4 to 5-fold higher in continuous flowsheets, this order of magnitude was negligible compared to the liquid waste in batch. The water PMI of Conti-ProA, Conti-ATPE and Conti-PP was 2 to 8-fold lower than Batch-ProA; therefore, these values were the key drivers for continuous environmental improvements.

A holistic approach considering not only the economic and PMI metrics derived from the model, but also the operational aspects of the different flowsheets was developed by using a multi-criteria decision-making technique. The rankings for the qualitative operational criteria were obtained from a survey sent to experts in the field and aggregate scores were generated for each strategy. The tool has predicted that Conti-ProA was the best option even when environmental or operational criteria were considered more important than cost savings. Also, when the relative importance of operational criteria was low, Conti-ATPE was the strategy with the lowest aggregate score across scenarios. A switch point in the decision, where ATPE would be preferable over product precipitation, was found when the importance of operational feasibility (e.g., robustness, ease of scale-up) outweighed the environmental aspect (ratio>0.7).

The final part of the chapter showed a target analysis to determine the cost reductions needed so the column-free capture flowsheets could meet a 15%

COG savings target compared to Conti-ProA. Based on ATPE and precipitation process parameters changes that showed a high impact in decreasing the cost of goods, the goal was to find the technological advances needed to justify the switch from protein A chromatography to aqueous two-phase extraction or product precipitation in mAb manufacturing. The installation of inline dilution and implementation of buffer concentrates would significantly benefit both column-free strategies. Also, the target was met when the usage of buffer concentrates was combined with increased perfusion volumetric productivities and increased harvest cell culture fluid percentage (HCCF %) in Conti-ATPE or the precipitates wash yield in Conti-PP. COG savings around 30% were possible at the most favourable conditions for both column-free capture flowsheets.

Chapter 4 explored the application of an LCA tool to carry out a more in-depth environmental evaluation of different mAb production flowsheets. When compared to the environmental PMI metrics derived from the process model, this tool allowed for a more comprehensive analysis of the entire life cycle of products, processes and activities, as the impact of energy consumption or raw materials extraction was part of the methodology. The LCA tool showed that Conti-ProA was the strategy also with the lowest carbon footprint amongst flowsheets. The absence of the CIP cleaning and the smaller processing train led to significant energy savings that were the key driver for the smaller environmental footprint. The high CO_2 emissions associated to PEG combined with the high energy consumption from the series of capture steps in Conti-PP were the main contributors for the high carbon footprint of the precipitation flowsheet. Also, contrary to what was observed through the PMI analysis, the environmental burden based on the carbon footprint of Conti-PP was higher than Batch-ProA. This result showed that different assessment methodologies (process mass intensity vs. product carbon footprint) can result in different conclusions. As PMI considers the quantity of generated waste, it favours intensified processes with lower water consumption, whereas PCF focuses on climate change and favours processes with lower GHG emissions. The framework was also used to understand the impact of process optimisation in each flowsheet's carbon emissions and showed significant PCF reductions

from continuous flowsheets after increasing cell culture productivity. Additionally, the analysis revealed that a higher degree of optimisation of Conti-PP, including increasing HCCF concentration, could result in carbon footprints lower than Conti-ProA. When converting the environmental savings into tangible day-to-day metrics, optimising the precipitation flowsheet led to a PCF reduction equivalent to reducing in more than 21 thousand the number of trees to offset mAb manufacture carbon emissions.

In **Chapter 5**, the current state-of-the-art and the view for the implementation of PAT in continuous bioprocesses was explored by conducting a survey and series of interviews with experts in the biopharma space. The pool of respondents was selected to include experts from different companies and departments (e.g., QCQA, Engineering, R&D), ensuring that the outcomes of the survey would reflect different perspectives and the industry as a whole. Detailed overall trends and opinions ranging from the most common factors slowing down PAT implementing to the predicted timelines for a widespread adoption of these technologies in mAb manufacturing were discussed. The survey results showed that 90% of the respondents had already integrated enhanced control in at least one processing step either during process development or manufacture. In addition, there was also a clear link drawn between PAT implementation and continuous manufacturing efforts. Specific process benefits deriving from the implementation of these technologies were discussed with the experts. Such information was crucial to understand the technical feasibility of PAT as well as determining the inputs for the process economics model. Nevertheless, the set of improvements applied to reflect PAT benefits was still limited, as the translation into economic impacts had only been done (either by the interviewed experts or found in the literature) for a few of these applications. In the process economics model, the changes to the base-case included USP parameters (e.g., increased perfusion volumetric productivities, increased perfusion rates), DSP parameters (e.g., increased resin capacity, increased membrane re-usage) and overall process benefits (e.g., decreased number of operators). The simulation tool predicted that the PAT attractiveness would depend on the production scale, with the investment being more diluted for medium and large scale facilities (500 and 1000 kg mAb

per year) compared to smaller scales (100 kg/year). The analysis was extended to look at the trade-offs in terms of levels of PAT investment and resulting process benefits (low vs. medium vs. high). The model determined that savings higher than 30% could be achieved at medium and large scales if the process performance was highly improved. The calculation of the number of production batches required so COG savings would balance the investment in PAT equipment also helped to provide clarity regarding the payback period of these technologies. For most of the scenarios assessed, the return would be visible in less than 1 year (10 batches). Overall, the results showed that mAb manufacture with end-to-end PAT integration (USP and DSP) would result in both COG savings and environmental gains, increasing the advantages of continuous bioprocessing.

6.2 Future work

While the chapters of results have showed how the framework was used to successfully simulate and evaluate continuous manufacturing processes, future work can further explore the capabilities of the decisional tool and deliver an expanded insight for a more informed decision-making when installing new processes and technologies.

In the process economics work, the economic potential of the different flowsheets was investigated on the basis that the target purity specifications were met for all capture techniques. However, impurity removal improvements can be necessary when using alternatives to ProA chromatography, thus, it can be of interest to incorporate these differences in the process assumptions and evaluate the economic impact of the diverse production outputs. On the other hand, advances on the technical performance of aqueous two-phase extraction and product precipitation were discussed with experts in the field who state that higher process yields and lower dilution levels are currently under investigation. Some of these improvements were included in Chapter 2, during the environmental optimisation of mAb flowsheets with product precipitation; however, the economic outcomes should also be studied, as it is expected an economic positive impact that may support these alternatives' business cases.

The decisional tool also embodied Monte Carlo simulations capabilities, where the flowsheets were evaluated under uncertainty and the likelihood of column-free techniques achieving lower costs than the continuous ProA reference was checked. These simulations were performed by fixing the production scale (e.g., 100, 500 or 1000 kg of mAb per year) and re-sizing the facilities according to the batch-to-batch variation of certain process parameters. However, it would also be relevant to assess the likelihood of these flowsheets to meet target product demands under uncertainty when the size of the facility is fixed. This study would further clarify the perceived risks associated with column-free capture strategies and characterise the output variability in pre-existing facilities that do not look for completely re-designing their production lines.

Furthermore, the framework developed in this research would also benefit from the integration of a risk-adjusted cash flow model in order to calculate net present value (NPV). As NPV is a metric which considers running costs, capital investment, but also future cash flows and risks, this would allow to account for longer-term consequences associated with implementing new production strategies.

In the environmental sustainability work, the carbon emissions associated with different mAb manufacturing flowsheets were evaluated as a way of quantifying the climate change potential of each strategy. This metric was the one highlighted in this work, as biopharma's net zero ambitions are mainly focused on the carbon footprint of bioprocesses. However, there are further impact categories, such as acidification potential, land use or ozone depletion, that can be analysed through the LCA tool. The relevance of these other metrics should be assessed together with industry experts to determine which factors must be in scope when looking at the environmental burden related to biomanufacturing.

To improve the confidence on the LCA outputs it would be also relevant to extend the current built-in databases to better represent biopharma-related raw materials and processes. One of the main challenges faced in this simulation software was the lack of environmental data for some critical components integrated in the production flowsheets, such as resins or filtration membranes. The necessary approximations were performed to support the assessment of

different raw materials' impact; however, future cooperation between suppliers and LCA users would certainly help increasing the robustness of this technique and provide more accurate estimations. On the other hand, also the application of sensitivity analysis in the input parameters, either raw materials or processes related, would offer a better understanding regarding the factors which have the largest influence on mAb production carbon footprint.

Although the presented LCA approach (cradle-to-gate) had in scope the disposal of solid waste (consumables: filtration membranes, single-use bags) via incineration, it would also be of interest to study the potential of energy recovery, which is currently not in scope. As the mAb production process is very energy intense, especially due to the HVAC systems and WFI preparation, the utilisation of the heat coming from waste disposal could decrease the facility energy demand and create a more sustainable scenario.

Comparing the energy outputs in this thesis with literature benchmarks showed that these calculations are highly dependent on the assumptions regarding HVAC and other ancillary activities requirements (e.g., WFI and CIP stations, media and buffer mixing). As the use-phase emissions are a big contributor for total product carbon footprint across flowsheets, accurate energy data is needed. Working with partners to get better HVAC data that covers fans, heating and cooling requirements for different area classifications would be highly recommended. Getting accurate data on current energy demands from WFI and CIP water generation and other process related activities would also be of relevance to increase the confidence in the energy outputs. Additionally, comparing current HVAC designs with more environmental-efficient future HVAC options could provide insights on the leads towards net zero manufacturing.

Finally, while the LCA tool presented in this research was only applied to mAb manufacturing to provide benchmarks on several mAb production strategies, it would be also useful to apply the framework to other modalities, such as CAR-T or AAVs. The current tool provides a strong foundation for the environmental assessment of other manufacturing bioprocesses, as most of the activities and processes can be transferred across schemes. This way, by deriving more

benchmarks for further modalities, one could provide a more complete understanding of the environmental impact of biopharmaceutical sector as a whole.

In the PAT evaluation work, the model captured current expected benefits of PAT related to yields, failure and costs. However, the full potential of PAT in an Industry 4.0 future requires further scoping and demonstration in industrial settings on what can be achieved with PAT enabling self-autonomous processes that adapt automatically to variability and ultimately facilitate real-time release. Then these need to be translated into clear process improvements with direct economic relevance that can be captured in the decisional tool to calculate the trade-offs between PAT investment and cost reduction.

Also, the impact of installing PAT on an environmental level should be assessed through PCF metric. While PMIs can easily be derived from the process economics model, a full life cycle assessment could also indicate the impact that the improved process performance coming from PAT installation could have on the carbon footprint of mAb production. As aforementioned, a sensitivity analysis in the LCA study would be helpful to understand the parameters influencing the most the environmental impact of mAb manufacturing. Therefore, USP (e.g., increased perfusion volumetric productivities) and DSP (e.g., lower buffer consumption) parameters influenced by enhanced control should integrate this analysis and provide another level of understanding on PAT gains.

As PAT implementation is also envisioned as a route to reduce process and product variability, the likelihood of meeting target costs should be compared with processes with higher uncertainty. Future studies should aim to integrate Monte Carlo simulations where the distribution of cost outputs of processes with and without PAT can be evaluated. Also, multiple interviewed experts have suggested the PAT integration in the process development stage, where these technologies can provide a faster process understanding and enable a more robust technology transfer into manufacturing. Thus, an NPV analysis would reflect the benefits of a shorter (and possibly less costly) development phase and time-to-market.

Chapter 7: Bibliography

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Appendix

A1. Chapter 3 appendix

A1.1 Mass balance equations

The input parameters and main model outputs of each stage are summarised in **Table A1.1**. Process variables such as *volume, mass, concentration* or *processing time* represent in/outputs of most operations and are not indicated.

Table A1.1 - Input parameters and key model outputs for each unit operation modelled with the tool.

Unit Operation	Mode	Technology	Class	Input parameters		Key model outputs
Inoculum	Batch/continuous	Vial/Shake flask	*Inoculum*	• Inoculation ratio		-
Cell culture	Batch	Fed-batch	*Bioreactor*	• Inoculation ratio • Target concentration	• Wet-cell volume	• Broth composition
	Continuous	Perfusion		• Volumetric productivity • ATF membrane capacity	• Perfusion rate • Wet-cell volume	• Broth composition • Flow-rate
Solid-Liquid Separation	Batch	Centrifugation	*Centrifugation*	• Operating duration limit • Solid carry-over	• Dewatering level • Wet-cell volume	• Yield • Supernatant composition
	Batch	Depth Filtration (dead-end)	*Filtration*	• Filter capacity • Flush volume	• Maximum Flux • Yield	• Membrane area • Number of filters
	Continuous	Depth filtration (cross-flow)		• Filter capacity • Flush volume	• Maximum Flux • Yield	• Membrane area • Number of filters
Purification	Batch/continuous	Chromatography	*Chromatography*	• Binding capacity • Number of cycles • Buffer volumes • Linear velocity	• Number of columns • Bed height • Resin loading tolerance • Yield	• Product stream composition • Column volume • Column Diameter
	Batch/continuous	Aqueous two-phase extraction	*Aqueous two-phase systems*	• Ratio of cells in • System composition	• Top/bottom phases ratio • Yield	• Product stream composition
	Batch/continuous	Precipitation	*Precipitation*	• Ratio of cells in • System composition	• Yield • Residence time	• Product stream composition
Viral Inactivation	Batch/continuous	(agitated tank)	*Reactor*	• Base/Acid volume		-
Virus removal filtration	Batch	Dead-end filtration	*Filtration*	• Filter capacity • Flush volume	• Maximum Flux • Yield	• Membrane area • Number of filters
	Continuous	Single-pass tangential flow filtration (SPTFF – cross flow)		• Filter capacity • Flush volume	• Maximum Flux • Yield	• Membrane area • Number of filters
Concentration and Diafiltration (UFDF)	Batch	Tangential flo filtration (TFF – cross flow)	*Filtration*	• Target concentration • Diafiltration cycles • Filter capacity	• Flush volume • Maximum Flux • Yield	• Concentration factor • Membrane area • Number of filters
	Continuous	SPTFF		• Target concentration • Diafiltration cycles • Filter capacity • Operating duration limit	• Flush volume • Maximum Flux • Yield	• Concentration factor • Membrane area • Number of filters

A1.1.1. Cell culture

$$m_{\frac{product}{batch}} = \frac{Demand}{\eta \times N_{batches}} \quad (A1.1)$$

$$BWV = \frac{m_{\frac{product}{batch}}}{Titre_{fed-batch\ or\ perfusion}} \quad (A1.2)$$

$$Titre_{perfusion} = \frac{VPR}{\mathrm{PR}} \quad (A1.3)$$

$$C_{out\ perfusion} = VPR \times d = \frac{m_{\frac{product}{batch}}}{V_{out}} \quad (A1.4)$$

$$V_{out\ perfusion} = BWV \times PR \times d \quad (A1.5)$$

$$V_{out\ fed-batch} = BWV \quad (A1.6)$$

$$Media_{fed-batch} = BWV \times (1 - i) \times (1 + Media\ Overfill) \quad (A1.7)$$

$$Media_{perfusion} = BWV \times \left[PR \times d + PR_{ramp-up} \times d_{ramp-up}\right] \times (1 + Media\ Overfill) \quad (A1.8)$$

$$V_{bioreactor} = \frac{BWV}{\mathrm{s}} \quad (A1.9)$$

$$N_{bioreactors} = \frac{V_{bioreactor}}{V_{available}} \quad (A1.10)$$

$$FR_{out\ perfusion} = \frac{BWV \times PR}{24} \quad (A1.11)$$

$m_{product/batch}$: Mass of product output per batch (kg/batch/year)

$Demand$: Annual product demand (kg)

η: Cumulative DSP yield (%)

$N_{batches}$: Number of batches designed per year (batch/year)

BWV: Bioreactor working volume (L)

$Titre$: Concentration of product in the moment of harvest (g/L installed)

VPR: Volumetric productivity (g/L/day)

PR: Perfusion rate, i.e. daily vessels harvested (BWVs/day)

$C_{out\ perfusion}$: Concentration of product collected after the entire perfusion time (g/L harvest)

d: Days of perfusion, excluding expansion phase (day)

$V_{out\ perfusion}$: Volume collected after the entire perfusion time (L harvest)

$V_{out\ fed-batch}$: Volume of product collected at once after a fed-batch batch (L harvest)

$Media$: Volume of media required for cell growing-out (L)

i: Inoculation ratio (%)

$Media\ overfill$: Safety factor of media fill-in to account for pipes/valves dead-volume (%)

$PR_{ramp-up}$: Perfusion rate, i.e. daily vessels harvested during the ramp-up phase (BWVs/day)

$V_{bioreactor}$: Actual bioreactor volume (L)

s: Vessel sizing safety factor (%)

$N_{bioreactor}$: Number of bioreactors needed for cell growing-out

$V_{available}$: Closest bioreactor size commercially available to the BWV (L)

$FR_{out\ perfusion}$: Flow-rate out of fermentation broth during perfusion (L/h)

A1.1.2 Centrifugation

$$FR_{in} = FR_{out} = \frac{V_{in}}{Duration\ limit} \quad (A.12)$$

$$m_{cells} = WCV \times V_{in} \quad (A.13)$$

$$m_{sediment} = m_{cell} \times (1 - SCO) \quad (A.14)$$

$$DW = \frac{m_{sediment}/\rho_{solid}}{V_{sediment} + m_{sediment}/\rho_{solid}} \Leftrightarrow V_{sediment} = \frac{m_{sediment}/\rho_{solid}}{DW} - m_{sediment}/\rho_{solid} \quad (A.15)$$

$$\eta_{centrifugation} = \frac{m_{in} - C_{in} \times V_{sediment}}{m_{in}} \times 100 \qquad (A.16)$$

V_{in}: Bioreactor working volume (BWV) from fermentation step

$Duration\ limit$: Limit set for the operation of centrifuge (h)

m_{cells}: Mass of cells found in the fermentation broth (kg)

WCV: Wet cell volume, i.e. ratio of cells found in the fermentation broth (%)

$m_{sediment}$: Mass of solid particles found in the sediment after centrifugation (kg)

SCO: Percentage of cell mass that stays in the supernatant (%)

DW: Dewatering level, i.e. level of liquid in the sediment (%)

ρ_{solid}: Density of solid phase in the sediment (kg/L)

$V_{sediment}$: Volume of liquid phase in the sediment (L)

$\eta_{centrifugation}$: Yield of centrifugation step (%)

A1.1.3 Filtration

Table A1.2 - Overview of filtration modelling equations

	Alternating tangential flow (ATF)	**Depth Filtration**		**Virus Removal Filtration**		**Concentration and Diafiltration**		
	Continuous	Batch	Continuos	Batch	Continuos	Batch	Continuos	
	Cross-flow	Dead-end	Cross-flow	Dead-end	Cross-flow	Cross-flow	Cross-flow	
Mass balance & Sizing	$A_{ATF} = \frac{FR_{in}}{Max\ flux}$ $FR_{out} = FR_{in}$ $m_{out} = m_{in} \times \eta$	$A_{DeF} = \frac{V_{in}}{Capacity_v}$ $m_{out} = m_{in} \times \eta$	$A_{VRF} = \frac{FR_{in}}{Max\ flux}$ $FR_{out} = FR_{in}$ $m_{out} = m_{in} \times \eta$	$A_{VRF} = \frac{m_{in}}{Capacity_m}$ $m_{out} = m_{in} \times \eta$	$A_{VRF} = \frac{FR_{in}}{Max\ flux}$ $FR_{out} = FR_{in}$ $m_{out} = m_{in} \times \eta$	$m_{out} = m_{in} \times \eta;\ CF = \frac{tC}{m_{out}/V_{in}}$ $P_{UF} = V_{in} \times (1 - 1/CF)$ $P_{DF} = V_{in}/CF \times DF_{cycles}$ $A_{UFDF} = \frac{(P_{UF} + P_{DF})/Max\ flux}{Duration\ limit}$	Ultrafiltration: $A_{ILC} = \frac{FR_{in}}{Max\ flux}$ $CF = \frac{tC}{\eta \times C_{in}}$ $FR_{out} = \frac{FR_{in}}{CF}$ $m_{out} = m_{in} \times \eta$	Diafiltration: $A_{ILD} = \frac{FR_{in}}{Max\ flux}$ $FR_{out} = FR_{in}$ $m_{out} = m_{in} \times \eta$
# units	$N_{filters} = \frac{V_{in}}{A_{ATF\ selected} \times Capacity_v}$	$N_{filters} = \frac{A_{DeF}}{A_{DeF\ available}}$		$N_{filters} = \frac{A_{DeF}}{A_{VRF\ available}}$		$N_{TFF\ skids} = \frac{A_{UFDF}}{A_{TFF\ available}}$	$N_{ILC\ skids} = \frac{A_{ILC}}{A_{ILF\ available}}$	$N_{ILD\ skids} = \frac{A_{ILD}}{A_{ILF\ available}}$
time	$Op_{time} = \frac{V_{in}}{FR_{in}}$	$Op_{time} = \frac{V_{in}}{A_{DeF} \times Max\ flux}$		$Op_{time} = \frac{V_{in}}{A_{VRF} \times Max\ flux}$	$Op_{time} = \frac{V_{in}}{FR_{in}}$	$Op_{time} = \frac{(P_{UF} + P_{DF})/Max\ flux}{A_{TFF\ available}}$	$Op_{time} = \frac{V_{in}}{FR_{in}}$	$Op_{time} = \frac{V_{in}}{FR_{in}}$

A_{ATF}: ATF filtering area required (m^2)

$A_{ATF\ selected}$: Closest filtering area commercially available to the A_{ATF} (m^2)

A_{DeF}: Depth filtering area required (m^2)

$A_{DeF\ available}$: Filtering area commercially available (m^2)

A_{ILC}: Area required for inline concentration (m^2)

A_{ILD}: Area required for inline diafiltration (m^2)

A_{UFDF}: Ultrafiltration area required (m^2)

A_{VRF}: Filtering area required for virus removal (m^2)

$Capacity_{v/m}$: Maximum loading capacity achievable by the filter according to vendor (L/m^2) / (g product/ m^2)

CF: Concentration factor

D_{cycles}: Diafiltration cycles

$Duration\ limit$: Limit set for the operation (h)

$FR_{in/out}$: Flow rate in/out (L/h)

$m_{in/out}$: Mass of product in/out (kg)

$Max\ flux$: Maximum flux achievable by the filter according to vendor (L/m^2/h)

$N_{filters}$: Number of filters needed with area available/selected

N_{skids}: Number of skids required

Op_{time}: Operating time (h)

P_{UF}: Ultrafiltration permeate (L)

P_{DF}: Diafiltration permeate (L)

tC: Target concentration (g/L)

$V_{in/out}$: Volume in/out (L)

η: Step yield (%)

A1.1.4 Chromatography

$$t_{non-loading} = \frac{BH}{LV_{non-loading}} \times \sum CV_{E/W/El/S/R/C} \quad (17)$$

$$CV = \frac{m_{in}}{DBC \times N_{col} \times N_{cycles}} \quad (18)$$

$$D = \sqrt{\frac{CV \times 4000}{\pi \times BH}} \quad (19)$$

$$R = \frac{N_{cycles} \times N_{batches}}{N_{lifespan\ cycles}} \quad (20)$$

$$RT_{loading} = \frac{BH}{LV_{loading}} \quad (21)$$

$$t_{loading} = RT_{loading} \times \frac{V_{in} / (N_{col} \times N_{cycles})}{CV} \quad (22)$$

CV: Chromatography column volume (L)

DBC: Dynamic binding capacity (kg product/L resin)

D: Chromatography column diameter (cm)

R: Replaces of resin during a year

$N_{lifespan\ cycles}$: Number of cycles corresponding to the resin lifespan

$t_{non-loading}$: Operating time of the non-loading chromatography steps (h)

BH: Chromatography column bed height (cm)

$LV_{non-loading}$: Linear velocity of non-loading steps (cm/h)

$CV_{E/W/El/S/R/C}$: Number of column volumes of buffer for each non-loading step

$RT_{loading}$: Residence time of product inside the column (h)

$LV_{loading}$: Linear loading velocity (cm/h)

$t_{loading}$: Operating time corresponding to loading step (h)

A1.1.5 Virus Inactivation

$$V_{out} = V_{in} \times (1 + Titrate_{acid}) \times (1 + Titrate_{base}) \quad (23)$$

$Titrate_{acid}$: Molar ratio of acid added for pH adjustment

$Titrate_{base}$: Molar ratio of base added for pH adjustment

A1.2 Equipment and material prices

Table A1.3 – Equipment costs and scaling factors used in the calculation of fixed capital investment (FCI)

Equipment	Base size	units	Base cost (USD)	Scaling factor, c
Bioreactor	200	L	240 000	0.38
SU Bioreactor Container	500	L	200 000	0.48
Incubator	24	#Flasks	5 000	NA
Hold-Tanks	500	L	50 000	0.38
Product Hold-Tanks	500	L	40 000	0.38
Product Accumulation-Tanks	500	L	40 000	0.38
Hold-Bags Container	500	L	7 000	1
Hold-Bags Trolleys	2000	L	450	0.13
Prep-Tanks	500	L	50 000	0.38
Chromatography Skid	3	L/min	160 000	0.25
PCC Skid	1.33	L/min	280 000	0.75
Chromatography Column	60	cm	66 500	0.90
Packing System	50	L/min	35 000	0.36
Centrifuge	600	L/hr	430 000	0.16
Filter Housing	2	sqm	3 500	0.31
TFF Skid	20	sqm	245 000	0.30
PW Vessel	1000	L	27 500	0.38
WFI Vessel	1000	L	36 500	0.38
Fill-Finish Machine-A	500	Vial/hr	350 000	1
ATF Filter Housing	5	sqm	90 300	-
ATF Skid	5	sqm	54 400	0.38
In-line filter skid	3.5	sqm	5 000	-
Continuous VI system	50	L	280 000	1
ATPE Extractor	30	cm	66 500	0.90
Precipitation Tubular Reactor	0.48	cm	1 000	0.30
SP TFF	0.33	sqm	250 000	-

Table A1.4 – Reagents and consumables prices

Material	Base size	units	Base cost (USD)	Scaling factor, c
Hold-Tank Guard Filter	1 000	L	310	0.65
Hold-Bags	500	L	530	0.47
Product Hold-Bags	500	L	530	0.47
Product Accumulation-Bags	500	L	530	0.47
Prep-Bags	500	L	530	0.47
Bioreactor Bags	500	L	5 100	0.44
Continuous VI bags	500	L	5 100	0.44
Wave Bioreactor	50	L	270	0.24
Shake Flask	0.50	L	500	0.40
ATF Membrane	5	sqm	10 300	0.35
Membrane Chromatography	0	L	180	0.55
Fill-Finish Syringe	10	mL	-	-
Vial	0.50	mL	10	-
0.45um Filter	0.60	sqm	340	-
Depth Filter	1	sqm	300	-
Virus Removal Membrane	1	sqm	6 800	-
Ultrafiltration Membrane	1	sqm	3 200	-
ILC - 0.065	0.07	sqm	4 177	-
ILC - 0.13	0.13	sqm	4 699	-
ILC - 0.7	0.70	sqm	9 399	-
ILC - 3.5	3.50	sqm	24 983	-
ILD - 0.11	0.11	sqm	15 574	-
ILD - 0.22	0.22	sqm	16 233	-
ILD - 1.2	1.20	sqm	22 669	-
SP TFF Membrane	1	sqm	4 243	-
Empty Pre-packed Column	1	cm	2 000	-
PEG 3350	1	Kg	2	-
Na-Phosphate	1	Kg	1	-
NaCl	1	Kg	0.2	-
NaOH	1	Kg	5	-
ZnCl2	1	Kg	0.20	-
HEPES	1	Kg	10	-
Glycine	1	Kg	3	-

A1.2 Multi-criteria decision making survey

Operational Criteria for Precipitation

Name:

Company/Institution:

Position held within the company/institution:

Survey Aim: Evaluate the operational attributes of promising column-free mAb capture techniques as substitutes of protein A chromatography in mAb manufacturing

This survey is composed of 2 questions. The survey should not take more than 10 min to be completed.

1. How would you rank the relative importance of the following criteria when developing mAb commercial scale processes?

   Please rank the criteria from A to E, **A being most important and E being the least important**.

Criteria	Rank
Robustness	
Ease of validation	
Ease of installation	
Ease of operation/ Degree of automation	
Ease of scale up	

2. For each criteria (i.e. row), how would you rate the performance of precipitation and protein A chromatography?

   Please rate the technologies from 1 to 5, **1 being a "low" score, 3 a "medium" score and 5 a "high" score.**

Criteria	Rating	
	ProA	**Precipitation**
Robustness		
Ease of validation		
Ease of installation		
Ease of operation/ Degree of automation		
Ease of scale up		

Figure A1.1 – Survey sent to industry and academia experts on precipitation operational criteria. The same survey was sent to evaluate aqueous-two phase extraction (ATPE)

Table A1.5 - Responses from operational criteria survey. R= respondent.

		ProA v. ATPE						ProA v. Precipitation										AVG ProA	STD ProA	AVG ATPE	STD ATPE	AVG PP	STD PP
		R1		R2		R3		R4		R5		R6		R7		R8							
		ProA	ATPE	ProA	ATPE	ProA	ATPE	ProA	PP	ProA	PP	ProA	PP	ProA	PP	ProA	PP						
Rating value, x_{ij}	**Robustness**	5	3	3	5	5	4	5	2	5	2	4	5	3	1	5	4	4.4	0.9	4.0	1.0	2.5	1.7
	Ease of validation	4	3	4	3	4	2	5	1	3	1	3	5	3	1	5	4	3.9	1.1	2.7	0.6	2.0	2.0
	Ease of installation	3	4	2	5	1	3	4	4	4	3	3	5	2	3	5	4	2.9	1.1	4.0	1.0	3.8	1.0
	Ease of operation	4	3	4	4	2	1	4	3	2	4	2	5	3	2	5	3	3.3	1.3	2.7	1.5	3.5	1.3
	Ease of scale up	4	4	4	5	3	5	5	3	1	5	2	5	3	2	5	3	3.4	1.8	4.7	0.6	3.8	1.5
Weight, E_i	**Robustness**	5		3		5		5		5		5		5		4		5					
	Ease of validation	4		5		3		2		2		4		4		3		3					
	Ease of installation	2		1		2		1		1		1		1		2		1					
	Ease of operation	4		2		1		3		4		2		2		1		2					
	Ease of scale up	4		4		4		4		3		3		3		3		4					

A2. Chapter 4 appendix

A2.1 Inputs of OpenLCA

Table A2.1 – Buffers' composition

Cell culture media	• Anhydrous Calcium Chloride: 0.165 g/L • Dextrose: 4.5 g/L • Magnesium Sulfate Anhydrous:0.1 g/L • Potassium Chloride: 0.33 g/L • Sodium Bicarbonate: 3 g/L • Sodium Chloride: 4.5 g/L • HEPES Buffer: 6 g/L • Sodium Phosphate 0.1 g/L • amino acids: 0.2 g/L
Diafiltration buffer	• 50 mM HEPES
Chromatography equilibration buffer	• 150mM NaCl • 50mM Tris
Chromatography wash buffer 1	• 1.8M $CaCl_2$ • 50mM Tris
Chromatography wash buffer 2	• 10mM NaCl • 50mM Tris
Chromatography elution buffer	• 10mM NaCl • 50mM glycine
Chromatography strip buffer	• 0.5M Sodium sulfate • 50mM NaOH
CIP buffer	• NaOH 1% w/v
General substitutions	• HEPES was substituted to methane sulfonic acid • Tris was substituted by dimeethylaminopropylamine

A2.2 Mass balances – 500 kg/year

Table A2.2 – Mass balance per batch using Batch-ProA (base case).

Step	Liquids	In (L)	Out (L)	Solids	In (g)	Out (g)
Seed	Media	875	80	Guardfilters	200	200
	CIP buffer	4037	4037	Bioreactors bags	0	0
	PW and WFI	2306	2307	Hold bags	0	0
	Buffer	0	0	Filters	0	0
				Resin	0	0
Bioreactor	Media	7879	716	Guardfilters	400	400
	CIP buffer	8163	8163	Bioreactors bags	0	0
	PW and WFI	4665	4665	Hold bags	0	0
	Buffer	0	0	Filters	0	0
				Resin	0	0
Centrifugation	Media	0	1008	Guardfilters	200	200
	CIP buffer	8944	8944	Bioreactors bags	0	0
	PW and WFI	3178	3178	Hold bags	0	0
	Buffer	0	0	Filters	0	0
				Resin	0	0
Depth filtration	Media	0	0	Guardfilters	200	200
	CIP buffer	4650	4650	Bioreactors bags	0	0
	PW and WFI	5057	4937 3	Hold bags	0	0
	Buffer	600	600	Filters	48000	48000
				Resin	0	0
ProA	Media	0	7071	Guardfilters	1400	1400
	CIP buffer	14677	14678	Bioreactors bags	0	0
	PW and WFI	8387	8387	Hold bags	0	0
	Buffer	20126	18920	Filters	0	0
				Resin	8294	8294
VI	Media	0	0	Guardfilters	400	400
	CIP buffer	4110	4110	Bioreactors bags	0	0
	PW and WFI	2348	2349	Hold bags	0	0
	Buffer	584	53	Filters	0	0
				Resin	0	0
CEX	Media	0	0	Guardfilters	1400	1400
	CIP buffer	14062	14063	Bioreactors bags	0	0
	PW and WFI	8035	8036	Hold bags	0	0
	Buffer	10201	11090	Filters	0	0
				Resin	9331	9331
AEX	Media	0	0	Guardfilters	1200	1200
	CIP buffer	12143	12143	Bioreactors bags	0	0
	PW and WFI	6938	6939	Hold bags	0	0
	Buffer	8335	7699	Filters	0	0
				Resin	9331	9331
VRF	Media		0	Guardfilters	200	200
	CIP buffer	2517	2517	Bioreactors bags	0	0
	PW and WFI	1738	1738	Hold bags	0	0
	Buffer	39	36	Filters	12000	12000
				Resin	0	0
UFDF	Media	0	0	Guardfilters	200	200
	CIP buffer	5102	5102	Bioreactors bags	0	0
	PW and WFI	4595	4595	Hold bags	0	0
	Buffer	8824	9478	Filters	48000	48000
	mAb	0	830	Resin	0	0

Table A2.3 – Conti-ProA (base case)

Step	Liquids	In (L)	Out (L)	Solids	In (g)	Out (g)
Seed	Media	130	12	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	4500	4500
	PW and WFI	0	0	Hold bags	2600	2600
	Buffer	0	0	Filters	0	0
				Resin	0	0
Bioreactor	Media	46112	10705	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	7000	7000
	PW and WFI	0	0	Hold bags	23600	23600
	Buffer	0	0	Filters	2000	2000
				Resin	0	0
ProA	Media		35526	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	0	0
	PW and WFI	0	0	Hold bags	31400	31400
	Buffer	24455	22266	Filters	0	0
				Resin	108000	108000
VI	Media	0	0	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	0	0
	PW and WFI	0	0	Hold bags	7580	7580
	Buffer	1060	96	Filters	0	0
				Resin	0	0
CEX	Media	0	0	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	0	0
	PW and WFI	0	0	Hold bags	22500	22500
	Buffer	11475	13107	Filters	0	0
				Resin	236000	236000
AEX	Media	0	0	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	0	0
	PW and WFI	0	0	Hold bags	13800	13800
	Buffer	7509	6455	Filters	0	0
				Resin	221000	221000
VRF	Media	0	0	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	0	0
	PW and WFI	500	500	Hold bags	0	0
	Buffer	50	50	Filters	20000	20000
				Resin	0	0
ILC	Media	0	0	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	0	0
	PW and WFI	46	46	Hold bags	0	0
	Buffer	65	1619	Filters	19	19
				Resin	0	0
ILD	Media	0	0	Guardfilters	0	0
	CIP buffer	0		Bioreactors bags	0	0
	PW and WFI	420	420	Hold bags	31000	31000
	Buffer	15984.92	15339	Filters	19	19
	mAb	0	1670	Resin	0	0

Table A2.4 – Conti-PP (base case)

Step	Liquids	In (L)	Out (L)	Solids	In (g)	Out (g)
Seed	Media	167	15	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	4500	4500
	PW and WFI	0	0	Hold bags	2600	2600
	Buffer	0	0	Filters	0	0
	PEG	0	0	Resin	0	0
Bioreactor	Media	59145	13731	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	7000	7000
	PW and WFI	0	0	Hold bags	24400	24400
	Buffer	0	0	Filters	2000	2000
	PEG	0	0	Resin	0	0
PP	Media	0	0	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	0	0
	PW and WFI	27340	0	Hold bags	84000	84000
	Buffer	0	0	Filters	0	0
	PEG	6370	0	Resin	0	0
SPTFF	Media	0	45566	Guardfilters	0	0
	CIP buffer	0		Bioreactors bags	0	0
	PW and WFI	2625	2625	Hold bags	23600	23600
	Buffer	42807	62823	Filters	2560	2560
	PEG	0	6370	Resin	0	0
RESOL	Media	0	0	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	0	0
	PW and WFI	0	0	Hold bags	7000	7000
	Buffer	3662	0	Filters	0	0
	PEG	0	0	Resin	0	0
Depth Filtration	Media	0		Guardfilters	200	200
	CIP buffer	3	3879	Bioreactors bags	0	0
	PW and WFI	3879	380	Hold bags	2600	2600
	Buffer	380	0	Filters	76000	76000
	PEG	0	0	Resin	0	0
ILC	Media	0	0	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	0	0
	PW and WFI	245	245	Hold bags	0	0
	Buffer	350	9192	Filters	19	19
	PEG	0	0	Resin	0	0
VI	Media	0	0	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	0	0
	PW and WFI	0	0	Hold bags	7240	7240
	Buffer	1037	94	Filters	0	0
	PEG	0	0	Resin	0	0
CEX	Media	0	0	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	0	0
	PW and WFI	0	0	Hold bags	22500	22500
	Buffer	11475	13040	Filters	0	0
	PEG	0	0	Resin	236000	236000
AEX	Media	0	0	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	0	0
	PW and WFI	0	0	Hold bags	13800	13800
	Buffer	7509	6455	Filters	0	0
	PEG	0	0	Resin	221000	221000
VRF	Media	0	0	Guardfilters	0	0

	CIP buffer	0	0	Bioreactors bags	0	0
	PW and WFI	500	500	Hold bags	1700	1700
	Buffer	50	50	Filters	20000	20000
	PEG	0	0	Resin	0	0
ILC	Media	0	0	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	0	0
	PW and WFI	46	46	Hold bags	0	0
	Buffer	65	1619	Filters	19	19
	PEG	0	0	Resin	0	0
ILD	Media	0	0	Guardfilters	0	0
	CIP buffer	0	0	Bioreactors bags	0	0
	PW and WFI	420	420	Hold bags	31000	31000
	Buffer	16066	15420	Filters	19	19
	PEG	0	0	Resin	0	0
	mAb	0	1670	-	-	-

A3. Chapter 5 appendix

A3.1 Survey

Process Analytical Technology (PAT) implementation in Continuous Bioprocessing

Name:

Company/Institution:

Position held within the company/institution:

Survey Aim: This survey is part of a UCL doctoral research project on process economics of continuous manufacture with PhD researcher Catarina Neves and supervisor Suzy Farid. We are seeking your input to gain insight into the impact of continuous bioprocessing on QCQA activities now and in the future where PAT is implemented to enable real-time release testing. This survey is composed of 8 questions and should take no longer than 10 min to be completed.

1. How would you rank the relative importance of the following benefits when considering PAT implementation? Please rank from 1-3, with 1 being the most important and 3 being the least important.

Reduce batch failure	
Higher overall yields or productivities (e.g. due to better control)	
Reduce offline QCQA costs	

Comments

2. Are there other key motivators for implementing PAT in continuous bioprocesses?

3. Where have you implemented PAT in you process (pilot or commercial scale)? Please feel free to elaborate on the types of PAT in the comments section. Please put an “X” in the appropriate row(s).

Cell culture	
Harvest	
Purification	
Other	
Not implemented	

Comments

4. Based upon your experience, what are the gaps slowing down the implementation of real-time release testing enabled by PAT in the mAb sector?

5. For continuous bioprocesses, when do you think we will see implementation of real-time release testing enabled by PAT in the mAb sector? Please put an "X" in the appropriate row.

< 1 year	
1 – 3 years	
4 – 5 years	
6 – 10 years	
> 10 years	
Not sure	

Comments

6. What would be a reasonable payback time (when savings coming from PAT implementation meet investment on PAT) that would make you consider investing in PAT? Please put an "X" in the appropriate row.

< 1 year	
1 – 3 years	
4 – 5 years	
6 – 10 years	
Not sure	

7. Based upon your experience, please indicate whether the equipment costs for the PAT systems below are in the right ballpark or too low or high (please do not consider data integration costs)? Scale: 2000 L perfusion bioreactor

	Lower than expected	**Roughly as expected**	**Higher than expected**
400k$ for Raman (system + probes)			
200k$ for Biocapacitance (system + probes)			
100k$ for each at-line HPLC system after ProA and polishing chrom. (excl. consumables)			
200k$ for each online LC-MS system (excl. consumables)			

Comments

8. Do you believe reagent and consumable costs from running PAT systems will significantly increase the total material costs for production?

Yes	
No	

Papers by the author

Neves, C. P. G., Coffman, J. L. and Farid, S. S. (2024) '**Evaluating end-to-end continuous antibody manufacture with column-free capture alternatives from economic, environmental, and robustness perspectives**', *Biotechnology Progress 2024*; e3427. doi: 10.1002/btpr.3427.

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1. For a research manuscript that has already been published (if not yet published, please skip to section 2)

a) **What is the title of the manuscript?**

Evaluating end-to-end continuous antibody manufacture with column-free capture alternatives from economic, environmental, and robustness perspectives

b) **Please include a link to or doi for the work**

DOI: 10.1002/btpr.3427

c) **Where was the work published?**

Biotechnology Progress – Bioseparations and Downstream Processing

d) **Who published the work?** (e.g. OUP)

Wiley

e) **When was the work published?**

January 2024

f) **List the manuscript's authors in the order they appear on the publication**

Catarina P. G. Neves; Jonathan L. Coffman; Suzanne S. Farid

g) **Was the work peer reviewed?**

Yes

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Yes

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Catarina P. G. Neves: Conceptualization; data curation; formal analysis; investigation; methodology; software; visualization; writing – original draft; writing – review and editing.

Jonathan L. Coffman: Conceptualization; resources; validation.

Suzanne S. Farid: Conceptualization; funding acquisition; project administration; resources; super- vision; validation; visualization; writing – review and editing.

4. **In which chapter(s) of your thesis can this material be found?**

Chapters 3 and 4

5. **e-Signatures confirming that the information above is accurate** (this form should be co-signed by the supervisor/ senior author unless this is not appropriate, e.g. if the paper was a single-author work)

Candidate

Catarina Neves

Date:

24/03/2024

Supervisor/ Senior Author (where appropriate)

Suzanne Farid

Date

24/03/2024