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Scale-Up of Monoclonal Antibody Production in CHO Cell Bioreactors: Process Optimisation Strategies

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ABSTRACT

Background: *Monoclonal antibodies (mAbs) produced in Chinese Hamster Ovary (CHO) cells dominate the biopharmaceutical pipeline, and moving a productive clone into a robust, high-titre manufacturing-scale bioreactor process remains a central engineering challenge.* ***Objective:*** *This article synthesises published evidence on strategies used to scale up CHO cell mAb production and optimise the resulting bioprocess.* ***Methods:*** *A narrative review of published studies on CHO cell bioprocess development, scale-up strategies, and process optimisation for mAb manufacturing, identified through PubMed, Google Scholar and Crossref searches, restricted to individually verified sources.* ***Findings:*** *Reported fed-batch titres have risen from tens of milligrams per litre in the mid-1980s to several grams per litre in contemporary processes , driven by host-cell engineering, feed-optimised media, and intensified seed-train strategies such as N-1 perfusion . Perfusion culture using alternating tangential flow or non-woven matrix retention sustains viable densities an order of magnitude above standard fed-batch . Scale-up relies on qualified scale-down models and high-throughput mini-bioreactors to translate lab findings to production vessels . Process analytical technology, including in-line Raman spectroscopy, increasingly enables real-time control of nutrients and critical quality attributes .* ***Conclusions:*** *Scale-up of CHO-based mAb manufacturing is best achieved through integrated cell-line, media, bioreactor-mode and analytical strategies validated at small scale within a quality-by-design regulatory framework.*

Keywords: *CHO cells; monoclonal antibody; bioreactor scale-up; fed-batch; perfusion culture*

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Abstract

Background: Monoclonal antibodies (mAbs) produced in Chinese Hamster Ovary (CHO) cells dominate the biopharmaceutical pipeline, and moving a productive clone into a robust, high-titre manufacturing-scale bioreactor process remains a central engineering challenge. **Objective:** This article synthesises published evidence on strategies used to scale up CHO cell mAb production and optimise the resulting bioprocess. **Methods:** A narrative review of published studies on CHO cell bioprocess development, scale-up strategies, and process optimisation for mAb manufacturing, identified through PubMed, Google Scholar and Crossref searches, restricted to individually verified sources. **Findings:** Reported fed-batch titres have risen from tens of milligrams per litre in the mid-1980s to several grams per litre in contemporary processes [1,2], driven by host-cell engineering, feed-optimised media, and intensified seed-train strategies such as N-1 perfusion [3,4,5]. Perfusion culture using alternating tangential flow or non-woven matrix retention sustains viable densities an order of magnitude above standard fed-batch [6,7]. Scale-up relies on qualified scale-down models and high-throughput mini-bioreactors to translate lab findings to production vessels [8,9,10]. Process analytical technology, including in-line Raman spectroscopy, increasingly enables real-time control of nutrients and critical quality attributes [11,12,13]. **Conclusions:** Scale-up of CHO-based mAb manufacturing is best achieved through integrated cell-line, media, bioreactor-mode and analytical strategies validated at small scale within a quality-by-design regulatory framework.

Keywords: CHO cells; monoclonal antibody; bioreactor scale-up; fed-batch; perfusion culture

Introduction

Chinese Hamster Ovary (CHO) cells are the predominant host for manufacturing therapeutic monoclonal antibodies because they support human-like post-translational modification, robust growth in suspension, and adaptation to chemically defined,

serum-free media [1,2]. Over four decades, volumetric productivity of CHO fed-batch cultures has increased roughly a hundredfold, from tens of milligrams per litre to several grams per litre, through combined advances in host cell line engineering, culture medium and feed design, and bioreactor process control [1,14]. Translating a laboratory-scale process delivering such titres into a robust manufacturing-scale operation, however, is non-trivial: mixing time, dissolved oxygen and pH gradients, shear, and nutrient/metabolite heterogeneity all change with vessel geometry and volume, and can compromise both yield and product quality if not anticipated during process development [15,10]. The bioprocessing industry has consequently been characterised as facing a persistent tension between rapidly rising titres and the capital, facility and analytical investment needed to manufacture reliably at commercial scale [16]. Historically, the field moved from serum-containing, batch-fed roller bottles to chemically defined, suspension-adapted fed-batch and perfusion bioreactors, a transition that underpins today's platform manufacturing processes for antibody-based biologics [1,14]. This article reviews published literature describing the principal strategies used to scale up CHO cell mAb bioprocesses and to optimise them at scale, spanning cell line and media development, bioreactor operating mode, scale-down modelling, process analytical technology, and quality/regulatory considerations.

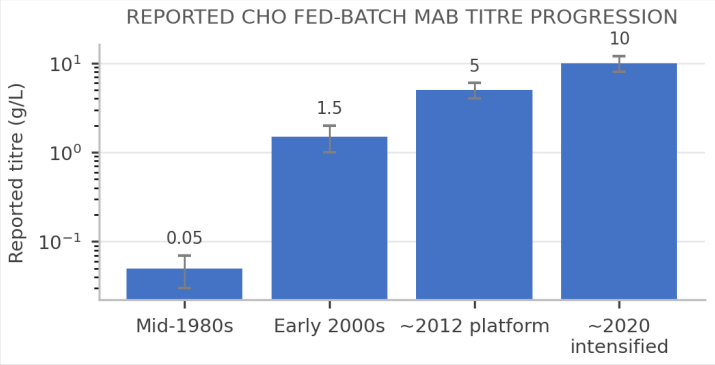
Methods

This is a narrative review of published studies on CHO cell bioprocess development, scale-up strategies, and process optimisation for monoclonal antibody manufacturing. Relevant literature was identified through structured searches of PubMed, Google Scholar and Crossref using combinations of the terms "CHO cell", "monoclonal antibody", "fed-batch", "perfusion", "bioreactor scale-up", "scale-down model", "process analytical technology" and "glycosylation". Original research articles, methodological reviews, and relevant regulatory guidance documents published in English were considered. Each candidate reference was individually verified against its primary bibliographic record (journal, volume, pages, and digital object identifier or PubMed identifier) before inclusion; sources that could not be fully verified were excluded. No new bioreactor experiments were performed for this article; all quantitative process data reported below are drawn from, and attributed to, the cited primary studies.

Cell Line Development and Media/Feed Optimisation

High-producing CHO clones are typically generated using glutamine synthetase or dihydrofolate reductase selection systems combined with targeted gene amplification and clone screening, yielding lineages capable of specific productivities that, together with process intensification, support gram-per-litre titres [2,3]. Attenuated glutamine synthetase selection markers increase selection stringency and improve the efficiency of isolating high-producing stable pools without extensive methionine sulfoximine amplification [3]. In parallel, chemically defined basal and feed media formulated with balanced amino acid, vitamin and trace-metal composition have replaced serum-supplemented formulations, improving lot-to-lot consistency and enabling regulatory-friendly, animal-component-free processes [14,17]. Iron content and speciation in feed media measurably affects both titre and product quality attributes such as charge variants, and optimising low-iron chemically defined feeds has been shown to improve titre while preserving formulated drug substance quality [4]. Feed timing, glucose and lactate control, and mild hypothermic temperature shifts late in culture are widely used to extend culture longevity and redirect metabolism toward productivity, though temperature shift can also alter antibody glycan profiles and must be balanced against quality targets [13,12]. Design-of-experiments approaches to medium composition, including for low-renewal-rate perfusion microbioreactors, are increasingly used to identify feed formulations that sustain both high density and consistent product quality [18]; targeted pH control strategies during fed-batch culture have similarly been shown to stabilise both titre and overall product quality [19]. Together, these host-cell and media strategies are generally combined rather than applied in isolation, since a clone's response to a given feed or temperature strategy is often cell-line specific and must be re-optimised whenever the production clone or medium platform changes [2,17].

Figure 1: Approximate progression of reported CHO fed-batch monoclonal antibody titres over process development eras, from tens of milligrams per litre in the mid-1980s to several grams per litre in intensified contemporary processes [1,2,5].



Bioreactor Operation Modes: Fed-Batch versus Perfusion and Scale-Up Engineering

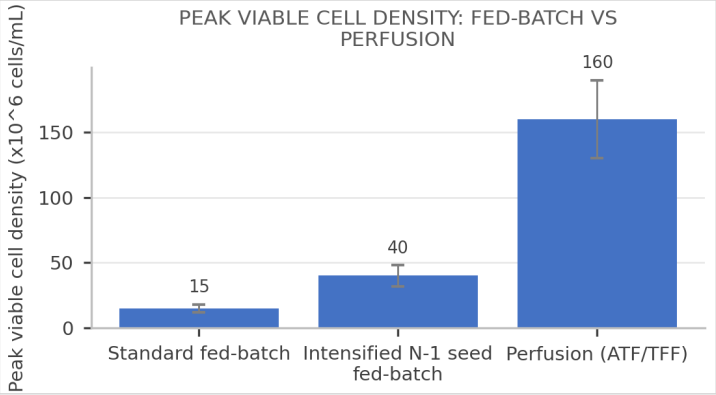
Fed-batch remains the dominant industrial mode, in which concentrated nutrient feeds are added to a batch culture to sustain growth and productivity over 10–14 days, and its scale-up is governed principally by matching oxygen transfer, mixing time and shear across vessel scales while adjusting the feeding regimen accordingly [17,10]. Perfusion culture, by contrast, continuously exchanges spent medium for fresh medium while retaining cells with devices such as alternating or tangential flow filtration, sustaining much higher viable cell densities and enabling smaller-footprint, higher-volumetric-productivity operations; densities on the order of 100–200×10⁶ cells/mL have been reported using WAVE bioreactor perfusion with alternating/tangential flow filtration, alongside antibody titres and product quality comparable to fed-batch [6,7]. Cell retention device choice materially affects achievable density and long-term process robustness, a consideration reviewed extensively for large-scale, high-density perfusion [15,20]; optimised perfusion strategies combining tailored feeding and bleed control have also been reported to stabilise volumetric productivity over extended culture durations [21]. Hybrid strategies that use an intensified, perfusion-fed N-1 seed train to inoculate a conventional fed-batch production bioreactor at very high seeding density have been reported to approximately double final fed-batch titres without requiring perfusion equipment in the production vessel itself [5,22]. Whichever mode is chosen, scale-up engineering must reconcile bioreactor hydrodynamics, agitation and sparging strategy with cell line-specific sensitivity, as demonstrated in

stirred-tank fed-batch scale-up studies varying hydrodynamic conditions and feeding regimen [10].

Table 1: Representative process optimisation strategies for CHO cell mAb bioreactor scale-up.

Strategy	Reported benefit	Key reference(s)
Attenuated GS selection marker	Higher selection stringency, faster isolation of high-producing clones	@lin2019
Low-iron chemically defined feed	Improved titre with maintained drug substance quality	@xu2018
N-1 perfusion intensified seed train	Approx. doubling of fed-batch production titre	@xu2020,@yongky2019
ATF/TFF perfusion culture	Peak viable cell density >100x10^6 cells/mL	@clincke2013a,@clincke2013b
Mild hypothermic temperature shift	Extended culture longevity, altered glycan profile	@sou2015

Figure 2: Reported peak viable cell densities for standard fed-batch, intensified N-1 seed fed-batch, and alternating/tangential-flow-filtration perfusion culture of CHO cells [6,7,5].



Process Analytical Technology and Scale-Down Models

Because production-scale experiments are costly and limited in number, qualified scale-down models are essential for process development and troubleshooting; high-throughput mini-bioreactor systems such as ambr®250 have been formally qualified against commercial-scale mAb processes, and shaken microbioreactor platforms matched by equal mixing time have been used to scale down and optimise CHO culture conditions with confidence that findings translate to larger vessels [8,9]. Within both scale-down and production vessels, process analytical technology increasingly supports real-time monitoring and feedback control: in-line Raman spectroscopy has been applied to monitor and control nutrient and metabolite concentrations and to support monoclonal antibody process intensification, reducing reliance on off-line sampling [11]. Such tools align with regulatory encouragement of process analytical technology and quality-by-design approaches to build quality into the process rather than testing it into the final product [23]. Continuous and semi-continuous operating strategies, including integrated continuous biomanufacturing, place additional demands on real-time control and have been the subject of dedicated technology roadmaps for the industry [24]. Scale-down qualification is typically demonstrated by showing that key process parameters, growth kinetics, titre and product quality attributes measured in the small-scale model fall within the historical variability of the large-scale process, providing the statistical basis on which development-scale experiments can be used to justify manufacturing-scale process changes [8,9].

Table 2: Scale-down and analytical tools supporting bioreactor scale-up decisions.

Tool	Purpose	Key reference(s)
ambr 250 mini-bioreactor	Qualified scale-down model for commercial-scale mAb processes	@manahan2019
Shaken microbioreactor (equal mixing time)	Scale-down and optimisation matched to production-scale mixing	@wiegmann2021
In-line Raman spectroscopy	Real-time nutrient/metabolite monitoring and control	@yilmaz2020
Cell retention device selection	Governs achievable density and robustness of perfusion scale-up	@voisard2003

Product Quality and Regulatory Considerations at Scale

Scale-up must preserve, not merely titre, but the critical quality attributes that determine antibody safety and efficacy, foremost among them N-linked glycosylation, which influences effector functions such as antibody-dependent cellular cytotoxicity and can shift with changes in dissolved oxygen, pH, temperature and feed composition that often accompany scale transitions [12,25]. Mild hypothermic temperature shifts, for example, can simultaneously raise titre and alter glycan galactosylation and fucosylation patterns, illustrating the need to track quality attributes, not only yield, throughout scale-up [13]. Disposable, single-use bioreactor systems have been widely adopted at both development and manufacturing scale because they reduce cleaning validation burden and turnaround time, but they introduce their own scale-dependent mixing and mass-transfer characteristics that must be characterised [26]. Overall, published experience indicates that successful, quality-preserving scale-up depends on early integration of analytical and regulatory science, consistent with quality-by-design principles, into cell line, media and bioreactor process development [23,27]. Regulatory expectations for biosimilar and originator mAbs alike require that critical quality attributes be defined, monitored and, where feasible, controlled across the full range of intended manufacturing scales, reinforcing the case for the scale-down and analytical strategies described above rather than relying solely on final product release testing [23,16].

Conclusion

Published literature converges on several complementary levers for scaling up CHO cell mAb production: engineered, high-producing cell lines and optimised chemically defined media; a deliberate choice between fed-batch, perfusion, or hybrid intensified seed-train operating modes; qualified scale-down models and high-throughput mini-bioreactors to de-risk technology transfer; and process analytical technology that enables real-time control of nutrients and quality attributes. No single strategy is universally superior; the optimal combination depends on product, cell line, and facility

constraints. Continued integration of these strategies within quality-by-design frameworks will be essential as the industry pursues both higher volumetric productivity and tighter, more consistent product quality at manufacturing scale.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Oja L conceived the review, conducted the literature search, and drafted the manuscript. Norkus R contributed to the literature screening and technical framing and critically revised the manuscript for important intellectual content. Rinaldi R contributed additional context and approved the final version for submission.

Ethics Statement

Not applicable. This is a narrative review of previously published literature and did not involve new experimental data, human participants, or animal subjects.

Data Availability

No new data were generated or analyzed for this review. All sources synthesized are cited in the reference list and are available through their original publishers.

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