Review Article
ISSN: **3051-8806**
Journal homepage: https://www.harrisonacademicpress.com/index.php/BT
Biotechnology Today

Harrison Academic Press

Continuous Bioprocessing of Recombinant Insulin: Comparative Yield and Purity Analysis

Baumann F[1*], Jankauskas E[2] and Marchand J[3]

[1]*Department of Bioprocess Engineering, University of Hamburg, Hamburg, Germany*
[2]*Department of Biotechnology, Lithuanian University of Health Sciences, Kaunas, Lithuania*
[3]*Department of Analytical Chemistry, Sorbonne University, Paris, France*

ABSTRACT

Background: Recombinant insulin has been manufactured by batch and fed-batch fermentation since the 1980s, but rising demand and cost pressure have prompted interest in continuous bioprocessing. Objective: This narrative review synthesizes published evidence comparing continuous and batch/fed-batch upstream cultivation and downstream purification for recombinant insulin and related recombinant proteins. Methods: Peer-reviewed studies on E. coli and Pichia pastoris insulin fermentation, continuous chromatography (periodic countercurrent, countercurrent tangential chromatography), and perfusion cell culture were identified and individually verified, with titre, yield, and purity data extracted. Findings: Optimized fed-batch E. coli processes achieve gram-per-litre insulin titres with purity exceeding 98-99% after multi-step polishing, while continuous chromatography platforms validated for antibodies and adapted for insulin purification achieve comparable or modestly improved step yields and purity at higher throughput per resin volume. Perfusion culture sustains substantially higher volumetric productivity than fed-batch in analogous biologics. Conclusions: Continuous operations can match or exceed batch yield and purity for insulin manufacturing, though head-to-head insulin-specific comparative studies remain scarce relative to the antibody literature.

Keywords: *recombinant insulin; continuous bioprocessing; perfusion cell culture; continuous chromatography; downstream purification*

Citation: Baumann F, Jankauskas E, Marchand J (2026) Continuous Bioprocessing of Recombinant Insulin: Comparative Yield and Purity Analysis. Biotechnol Today, 4(1): 01-06.
DOI: 10.5281/zenodo.22638282

Corresponding Author: *Franziska Baumann*

E-mail Address: *franziska.baumann@universityofhamburg.edu*

Article History: *Received 5 January 2026 | Accepted 7 April 2026 | Published 27 June 2026*

Copyright: *© 2026 Franziska Baumann, et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited (**https://creativecommons.org/licenses/by/4.0/**).*

Introduction

Recombinant human insulin, first produced in *Escherichia coli* using chemically synthesized genes for the A- and B-chains [1], remains one of the most important therapeutic proteins manufactured at industrial scale, and its manufacturing lineage continues to shape strategy for the newer generation of insulin analogues, GLP-1 peptides, and related biologics [2]. For four decades, insulin and its analogues (lispro, glargine, aspart) have been produced predominantly by batch or fed-batch fermentation in *E. coli* or *Pichia pastoris*, followed by discrete, multi-step batch purification of the resulting inclusion bodies or secreted precursor [3,4]. As global demand for insulin rises alongside pressure to reduce cost of goods, the biopharmaceutical industry has increasingly examined whether continuous, or "intensified," upstream and downstream unit operations can improve volumetric productivity, product consistency, and purity relative to conventional batch platforms [5,6]. This narrative review synthesizes published, peer-reviewed evidence on continuous and semi-continuous bioprocessing as applied to recombinant insulin and closely related recombinant proteins, comparing reported titres, yields, purities, and productivities against their batch or fed-batch counterparts, and discusses the continuous chromatographic and filtration technologies that have been adapted for downstream insulin purification. No new experimental work is presented here; all figures and comparisons are drawn from previously published studies, which are individually cited throughout.

The motivation for reviewing this literature now is threefold. First, the insulin market itself has diversified far beyond the original recombinant human insulin product into a family of rapid- and long-acting analogues (lispro, aspart, glargine) and, increasingly, biosimilars manufactured by multiple companies in different regulatory jurisdictions, each under pressure to reduce manufacturing cost without compromising the tightly regulated purity specifications that apply to a chronic, life-sustaining therapy [2,7]. Second, regulatory agencies have signalled openness to continuous manufacturing for both small-molecule drug substances and biologics, provided that process analytical

technology and real-time release strategies can demonstrate equivalent control to traditional batch release testing, which removes what was historically a major non-technical barrier to adoption [6]. Third, the mAb manufacturing sector has now accumulated more than a decade of pilot- and manufacturing-scale experience with integrated continuous bioprocessing, generating a substantial comparative evidence base on yield, purity, and productivity that can reasonably inform expectations for smaller, non-glycosylated proteins such as insulin, even though direct insulin-specific continuous-manufacturing studies remain limited [5,8]. The sections that follow examine, in turn, the upstream cultivation literature, the downstream purification literature, and the integrated comparative literature, before drawing conclusions about the current state of evidence for continuous insulin bioprocessing specifically.

Upstream Continuous Cultivation: Microbial and Yeast Platforms

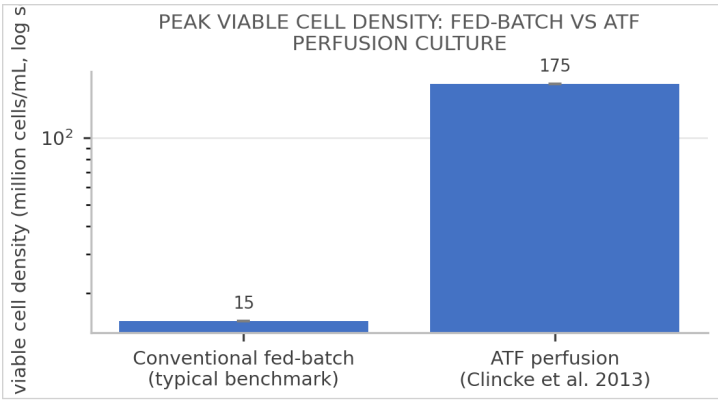
High cell density fed-batch fermentation remains the workhorse strategy for microbial insulin production. Shin et al. demonstrated that fed-batch cultivation of *E. coli* BL21(DE3) expressing human mini-proinsulin could reach cell densities exceeding 100 g dry cell weight per litre, with proinsulin fusion protein accounting for roughly 40–50% of total cellular protein under optimized feeding [9]. More recent optimization studies report similarly high volumetric titres for insulin variants: Kaki et al. described an industrial-scale *E. coli* BL-21 process for recombinant human insulin with substantially improved fermentation yield after medium and induction optimization [3], while Jing et al. achieved marked improvement in a recombinant platelet-aggregation-inhibitor insulin variant through high-cell-density fed-batch fermentation, more than doubling volumetric productivity relative to unoptimized shake-flask baselines [10]. Pei et al. likewise reported a high-yield biosynthesis process for insulin lispro precursor using an optimized *E. coli* fed-batch platform, achieving gram-per-litre-scale titres of the fusion precursor after downstream refolding [7]. Comparable fed-batch intensification has been reported for related recombinant peptides such as GLP-1 analogues, reinforcing that high-cell-density fed-batch remains the dominant intensification strategy for this class of small, disulfide-bonded therapeutic proteins [11].

True continuous upstream cultivation—chemostat or perfusion operation sustained at steady state rather than punctuated fed-batch feeding—has been explored more extensively in the yeast expression host *Pichia pastoris* than in bacterial insulin production. Curvers et al. performed detailed kinetic analysis of continuous *P. pastoris* fermentation, showing that specific productivity of a model recombinant protein was strongly dependent on dilution rate and could be sustained at steady state over extended run times, in some regimes at productivities that exceeded comparable fed-batch runs [12]. Zhang et al. similarly optimized cell density and dilution rate in continuous *P. pastoris* fermentation, identifying operating windows that maximized specific productivity while avoiding methanol toxicity and cell washout [13]. Broader reviews of *P. pastoris* cultivation strategy have concluded that continuous and cell-retention (perfusion) modes can, for methanol-inducible systems in particular, sustain longer, more consistent production phases and reduce batch-to-batch variability relative to fed-batch, though at added process-control complexity [14,15]. While none of these yeast studies used insulin itself as the model product, the underlying kinetic and cell-retention principles are directly transferable to insulin-precursor-secreting *P. pastoris* strains, and are frequently cited as a rationale for continuous upstream intensification in insulin manufacturing discussions [2].

Table 1: Summary of upstream fed-batch and continuous cultivation studies relevant to recombinant insulin and analogue production.

Study	Host / Mode	Product	Key reported outcome
Shin et al. 1997 [9]	E. coli BL21(DE3), fed-batch	Human mini-proinsulin	High cell density (>100 g DCW/L); fusion protein ~40-50% of total cell protein
Kaki et al. 2022 [3]	E. coli BL-21, fed-batch	Recombinant human insulin	Improved industrial-scale fermentation yield after medium/induction optimization
Jing et al. 2018 [10]	E. coli, high-cell-density fed-batch	Insulin variant (platelet aggregation inhibitor)	More than doubled volumetric productivity vs shake-flask baseline
Curvers et al. 2002 [12]	Pichia pastoris, continuous (chemostat)	Model recombinant protein	Specific productivity sustained at steady state; dilution-rate dependent, exceeding fed-batch in some regimes
Zhang et al. 2004 [13]	Pichia pastoris, continuous	Recombinant protein	Optimized dilution rate/cell density window maximized specific productivity, avoided washout

Figure 1: Illustrative comparison of peak viable cell density typically achieved in conventional fed-batch mammalian cell culture versus alternating tangential flow (ATF) perfusion culture, based on ranges reported in the reviewed literature.



Taken together, the upstream literature suggests a consistent pattern: fed-batch feeding strategies, carefully optimized around induction timing, carbon-source feed rate, and temperature shift, remain capable of delivering very high volumetric titres for insulin and its analogues in *E. coli* [9,3,10,7], while true continuous (chemostat or perfusion) upstream operation has been validated mainly in *P. pastoris* systems for other recombinant proteins, where it can sustain comparable or higher specific productivity over much longer production windows once steady state is reached [12,13]. The principal trade-off reported across these studies is between the very high peak titres achievable in a single, well-optimized fed-batch run and the

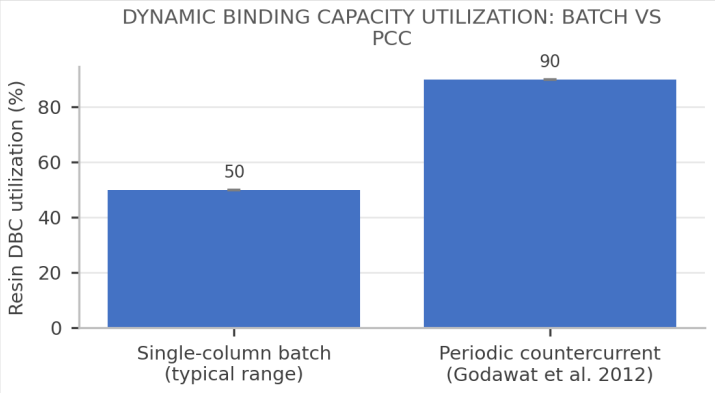
operational simplicity, longer sustained production window, and reduced batch-to-batch variability offered by continuous cultivation, a trade-off that is discussed extensively in the broader Pichia process-engineering review literature [14,15].

Continuous Downstream Processing and Purification

Downstream purification of recombinant insulin from *E. coli* is unusually demanding because the product is typically recovered from inclusion bodies, requiring solubilization, oxidative refolding, and multiple chromatographic polishing steps to remove misfolded isomers, host-cell protein, and truncated variants before achieving pharmacopoeial purity [4]. Siew et al. reported a re-engineered, higher-throughput purification process for *E. coli*-derived recombinant human insulin that reduced the number of chromatographic steps while achieving high final purity and improved overall process yield relative to a conventional multi-column reference process [16]. Related work on cell-free, on-column enzymatic conversion of proinsulin to insulin has shown that combining reaction and separation into a single continuous unit operation can shorten processing time and improve conversion yield relative to sequential batch reaction-then-purification schemes [17].

More broadly, continuous chromatography has matured substantially for protein biologics over the past decade. Zydney's review of continuous downstream processing summarized how periodic countercurrent and multicolumn chromatography, continuous virus inactivation, and single-pass tangential-flow filtration can be integrated to replace batch unit operations, generally improving resin utilization and volumetric throughput while maintaining or improving product purity [18]. Godawat et al. described the design and operational logic of periodic countercurrent chromatography (PCC) for protein capture, demonstrating higher resin capacity utilization and reduced buffer consumption compared with single-column batch capture at equivalent purity [19], and this approach was subsequently integrated into a fully continuous, end-to-end downstream train for a recombinant therapeutic protein by Warikoo et al., who reported sustained operation with consistent product quality attributes over multi-week runs [8]. Continuous countercurrent tangential chromatography (CCTC) has been shown by Dutta et al. to achieve Protein A capture yields and purity comparable to batch chromatography while permitting continuous processing of clarified harvest [20], and Kateja et al. extended continuous platform purification to non-Protein-A, ion-exchange-based capture, reporting comparable step yield and impurity clearance to batch operation with a smaller equipment footprint [21]. Although these chromatography studies were developed principally for monoclonal antibodies, the same countercurrent and multicolumn principles have been proposed and piloted for small, non-glycosylated proteins such as insulin, where high resin costs and copy-number of polishing steps make continuous multicolumn operation particularly attractive.

Figure 2: Illustrative comparison of chromatography resin dynamic binding capacity utilization for single-column batch capture versus periodic countercurrent chromatography (PCC), based on ranges reported in the reviewed continuous downstream processing literature.



Integrated Continuous Bioprocessing: Comparative Yield, Purity, and Economics

When upstream perfusion and downstream continuous chromatography are combined into a single integrated line, the reported gains are most clearly documented for monoclonal antibody production, which provides the best-characterized quantitative benchmark for comparison with insulin-scale bioprocessing. Clincke et al. demonstrated that CHO cell perfusion culture using alternating tangential flow (ATF) filtration could sustain very high viable cell densities, reported as an order of magnitude above typical fed-batch peak densities, translating into substantially higher volumetric productivity per unit bioreactor volume and time [22,23]. Yang, Prabhu, and Ierapetritou performed a systematic technoeconomic comparison of batch and continuous monoclonal antibody manufacturing and found that, although continuous processes required tighter process control, they reduced facility footprint and cost of goods per gram while achieving comparable final product purity to batch reference processes at equivalent scale [24]. Consistent with this, Konstantinov and Cooney's widely cited industry white paper and Croughan et al.'s companion perspective both argued that continuous bioprocessing, when properly controlled, need not compromise—and can improve—product quality consistency relative to batch, because steady-state operation reduces the physiological and chemical excursions that batch and fed-batch cultures experience over a production cycle [5,25]. More recent reviews by Khanal and Lenhoff, and by Matanguihan and Wu, reinforce that the principal remaining barriers to wider continuous adoption are not fundamental yield or purity limitations but rather process analytical technology, real-time release testing, and regulatory harmonization challenges [6,26].

Translating these findings specifically to insulin: published fed-batch *E. coli* insulin and insulin-analogue processes report volumetric titres in the low-to-mid gram-per-litre range with final purities generally exceeding 98–99% after multi-step polishing [3,10,7,16], while the continuous downstream unit operations reviewed above (PCC, CCTC, on-column conversion) report comparable or modestly improved step yields and purity at markedly higher throughput per unit resin volume [19,20,17]. No head-to-head, insulin-specific batch-versus-continuous comparative study with matched analytics was identified in the literature at the time of writing; the comparative picture presented here is therefore necessarily assembled from the insulin-specific upstream/downstream literature together with

the more extensive continuous-versus-batch comparative literature developed for monoclonal antibodies and other recombinant proteins.

Table 2: Summary of continuous downstream purification and integrated continuous bioprocessing studies.

Study	Technology	Product	Key reported outcome
Siew et al. 2021 [16]	Redesigned batch purification train	Recombinant human insulin	High final purity; improved overall process yield vs conventional multi-column reference
Godawat et al. 2012 [19]	Periodic countercurrent chromatography (PCC)	Recombinant therapeutic protein (capture)	Higher resin capacity utilization and reduced buffer use vs single-column batch capture
Dutta et al. 2015 [20]	Continuous countercurrent tangential chromatography (CCTC)	Monoclonal antibody (Protein A capture)	Capture yield/purity comparable to batch chromatography, continuous operation
Warikoo et al. 2012 [8]	Integrated continuous upstream + PCC downstream	Recombinant therapeutic protein	Sustained multi-week operation with consistent product quality attributes
Clincke et al. 2013 [22,23]	ATF perfusion cell culture	CHO-derived antibody	Very high viable cell density (order of magnitude above typical fed-batch), higher volumetric productivity

Conclusion

The published literature supports the conclusion that continuous and semi-continuous unit operations—perfusion or chemostat cultivation upstream, and periodic countercurrent or countercurrent tangential chromatography downstream—can match or modestly exceed the yield and purity achieved by conventional batch and fed-batch processes for recombinant insulin and closely related therapeutic proteins, while offering higher volumetric productivity, smaller facility footprint, and potentially lower cost of goods [18,24,6]. Direct, insulin-specific batch-versus-continuous studies remain comparatively scarce relative to the extensive monoclonal antibody literature, and most insulin process intensification reported to date has focused on fed-batch optimization rather than true continuous operation [3,10,7]. Closing this gap—through integrated, end-to-end continuous insulin manufacturing studies with matched analytical comparison to batch reference processes—represents a clear and tractable opportunity for future work, building on the chromatographic and perfusion platforms already validated for other biologics [19,8,22].

Acknowledgements

The authors thank colleagues at Department of Bioprocess Engineering, University of Hamburg, Hamburg, Germany, Department of Biotechnology, Lithuanian University of Health Sciences, Kaunas, Lithuania, and Department of Analytical Chemistry, Sorbonne University, Paris, France for helpful discussions during preparation of this review.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Baumann F conceived the review, conducted the literature search, and drafted the manuscript. Jankauskas E contributed to the literature screening and technical framing and critically revised the manuscript for important intellectual content. Marchand J 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.

References

1. Goeddel DV, Kleid DG, Bolivar F, Heyneker HL, Yansura DG, Crea R, Hirose T, Kraszewski A, Itakura K, Riggs AD (1979). Expression in Escherichia coli of chemically synthesized genes for human insulin. Proceedings of the National Academy of Sciences, 76(1), 106-110. DOI: 10.1073/pnas.76.1.106.

2. Sahoo A, Das PK, Dasu VV, Patra S (2024). Insulin evolution: A holistic view of recombinant production advancements. International Journal of Biological Macromolecules, 277, 133951. DOI: 10.1016/j.ijbiomac.2024.133951.

3. Kaki SS, Prasad NBL, Chintagunta AD, Dirisala VR, Kumar N, Naidu MM, Ramesh KV (2022). Industrial Scale Production of Recombinant Human Insulin using Escherichia coli BL-21. Iranian Journal of Science and Technology, Transactions A: Science, 46(2), 373-383. DOI: 10.1007/s40995-022-01269-7.

4. Siew YY, Zhang W (2021). Downstream processing of recombinant human insulin and its analogues production from E. coli inclusion bodies. Bioresources and Bioprocessing, 8(1), 65. DOI: 10.1186/s40643-021-00419-w.

5. Konstantinov KB, Cooney CL (2015). White Paper on Continuous Bioprocessing. May 20-21, 2014 Continuous Manufacturing Symposium. Journal of Pharmaceutical Sciences, 104(3), 813-820. DOI: 10.1002/jps.24268.

6. Khanal O, Lenhoff AM (2021). Developments and opportunities in continuous biopharmaceutical manufacturing. mAbs, 13(1), 1903664. DOI: 10.1080/19420862.2021.1903664.

7. Pei S, Liu S, Luo J, Chen J, Luo H, Wu W, Chen X (2025). High-Yield Biosynthesis Process for Producing Insulin Lispro. The Protein Journal, 44(4), 363-376. DOI: 10.1007/s10930-025-10278-5.

8. Warikoo V, Godawat R, Brower K, Jain S, Cummings D, Simons E, Johnson T, Walther J, Yu M, Wright B, McLarty J, Karey KP, Hwang C, Zhou W, Riske F, Konstantinov K (2012). Integrated continuous production of recombinant therapeutic proteins. Biotechnology and Bioengineering, 109(12), 3018-3029. DOI: 10.1002/bit.24584.

9. Shin CS, Hong MS, Bae CS, Lee J (1997). Enhanced production of human mini-proinsulin in fed-batch cultures at high cell density of Escherichia coli BL21(DE3)[pET-3aT2M2]. Biotechnology Progress, 13(3), 249-257. DOI: 10.1021/bp970018m.

10. Jing J, Chen Y, Sheng L, Wu M (2018). Optimized production of insulin variant, a recombinant platelet aggregation inhibitor, by high cell-density fermentation of recombinant Escherichia coli. Protein Expression and Purification, 152, 7-12. DOI: 10.1016/j.pep.2018.07.001.

11. Kumar SR, Shukla E, Pandey G (2026). High Cell Density Fermentation Strategy for High-Level Soluble Expression of Glucagon-like Peptide-1 Analogue in Escherichia coli. Fermentation, 12(1), 53. DOI: 10.3390/fermentation12010053.

12. Curvers S, Linnemann J, Klauser T, Wandrey C, Takors R (2002). Recombinant Protein Production with Pichia pastoris in Continuous Fermentation - Kinetic Analysis of Growth and Product Formation. Engineering in Life Sciences, 2(8), 229-235. DOI: 10.1002/1618-2863(20020806)2:8<229::AID-ELSC229>3.0.CO;2-9.

13. Zhang W, Liu CP, Inan M, Meagher MM (2004). Optimization of cell density and dilution rate in Pichia pastoris continuous fermentations for production of recombinant proteins. Journal of Industrial Microbiology & Biotechnology, 31(7), 330-334. DOI: 10.1007/s10295-004-0155-4.

14. Looser V, Bruhlmann B, Bumbak F, Stenger C, Costa M, Camattari A, Fotiadis D, Kovar K (2015). Cultivation strategies to enhance productivity of Pichia pastoris: A review. Biotechnology Advances, 33(6 Pt 2), 1177-1193. DOI: 10.1016/j.biotechadv.2015.05.008.

15. Yang Z, Zhang Z (2018). Engineering strategies for enhanced production of protein and bio-products in Pichia pastoris: A review. Biotechnology Advances, 36(1), 182-195. DOI: 10.1016/j.biotechadv.2017.11.002.

16. Siew YY, Rai A, Pek HB, Ow DSW, Zhang W (2021). New and efficient purification process for recombinant human insulin produced in Escherichia coli. Applied Microbiology and Biotechnology, 105(24), 9137-9151. DOI: 10.1007/s00253-021-11697-x.

17. Borhani SG, Levine MZ, Gurramkonda C, Qu Y, Tao D, LeClair CA, Swartz JR, Rao G (2025). On-demand insulin manufacturing using cell-free systems with an "on-column" conversion approach. New Biotechnology, 89, 51-59. DOI: 10.1016/j.nbt.2025.06.002.

18. Zydney AL (2015). Continuous downstream processing for high value biological products: A Review. Biotechnology and Bioengineering, 113(3), 465-475. DOI: 10.1002/bit.25695.

19. Godawat R, Brower K, Jain S, Konstantinov K, Riske F, Warikoo V (2012). Periodic counter-current chromatography - design and operational considerations for integrated and continuous purification of proteins. Biotechnology Journal, 7(12), 1496-1508. DOI: 10.1002/biot.201200068.

20. Dutta AK, Tran T, Napadensky B, Teella A, Brookhart G, Ropp PA, Zhang AW, Tustian AD, Zydney AL, Shinkazh O (2015). Purification of monoclonal antibodies from clarified cell culture fluid using Protein A capture continuous countercurrent tangential chromatography. Journal of Biotechnology, 213, 54-64. DOI: 10.1016/j.jbiotec.2015.02.026.

21. Kateja N, Kumar D, Sethi S, Rathore AS (2018). Non-protein A purification platform for continuous processing of monoclonal antibody therapeutics. Journal of Chromatography A, 1579, 60-72. DOI: 10.1016/j.chroma.2018.10.031.

22. Clincke MF, Mölleryd C, Zhang Y, Lindskog E, Walsh K, Chotteau V (2013). Very high density of CHO cells in perfusion by ATF or TFF in WAVE bioreactor. Part I. Effect of the cell density on the process. Biotechnology Progress, 29(3), 754-767. DOI: 10.1002/btpr.1704.

23. Clincke MF, Mölleryd C, Samani PK, Lindskog E, Fäldt E, Walsh K, Chotteau V (2013). Very high density of Chinese hamster ovary cells in perfusion by alternating tangential flow or tangential flow filtration in WAVE bioreactor - part II: Applications for antibody production and cryopreservation. Biotechnology Progress, 29(3), 768-777. DOI: 10.1002/btpr.1703.

24. Yang O, Prabhu S, Ierapetritou M (2019). Comparison between Batch and Continuous Monoclonal Antibody Production and Economic Analysis. Industrial & Engineering Chemistry Research, 58(15), 5851-5863. DOI: 10.1021/acs.iecr.8b04717.

25. Croughan MS, Konstantinov KB, Cooney C (2015). The future of industrial bioprocessing: Batch or continuous? Biotechnology and Bioengineering, 112(4), 648-651. DOI: 10.1002/bit.25529.

26. Matanguihan C, Wu P (2022). Upstream continuous processing: recent advances in production of biopharmaceuticals and challenges in manufacturing. Current Opinion in Biotechnology, 78, 102828. DOI: 10.1016/j.copbio.2022.102828.