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REVIEW

Bioprocess Engineering: Advances in Cell Culture Systems, Reactor Design, Scale-up Strategies, and Intensification Processes for the Production of Biological and Bioactive Compounds

Ingeniería de Bioprocesos: Avances en Sistemas de Cultivo Celular, Diseño de Reactores, Estrategias de Escalado y Procesos de Intensificación para la Producción de Compuestos Biológicos y Bioactivos

Freddy Ednildon Bautista-Vanegas[1,2], Pablo Carías[3], Luis Mariano Tecuatl Gómez[4], Rebeca Rocio Gomez Rosales[5], Themis Karim Ocampo Gamboa[6], Jhossmar Cristians Auza-Santivañez[7], Estefannie Eunice Valverde Fernández[8], Blas Apaza-Huanca[7], Ingrid Neysa Cabezas-Soliz[7], Jose Luis Diaz-Guerrero[9], Ruben Marco Antonio Soliz-Mendoza[10]

[1]Kliniken Beelitz GmbH Neurologische Rehabilitationsklinik: Beelitz Heilstätten, Brandenburg, DE. Germany.
[2]Neurologisches Fachkrankenhaus für Bewegungsstörungen / Parkinson: Beelitz Heilstätten, Brandenburg, DE. Germany.
[3]Facultad de Ciencias Médicas. Departamento de Cirugía. Universidad Nacional Autónoma de Honduras. Tegucigalpa, Honduras.
[4]ISSSTE San José del Cabo. Universidad De Los Cabos. México.
[5]Hospital Infanto Juvenil Menino de Jesus M. São Paulo, Brazil.
[6]Universidad de Aquino Bolivia (UDABOL). Santa Cruz, Bolivia.
[7]Ministerio de Salud y Deportes. Instituto Académico Científico Quispe-Cornejo. La Paz, Bolivia.
[8]Universidad Evangélica Boliviana. Sociedad Científica de Estudiantes de Medicina SOCIEM-UEB. Santa Cruz, Bolivia.
[9]Seguro Social Universitario Tarija (SSUT). Servicio de Ecografía. Tarija, Bolivia.
[10]Centro de Salud "San Juan Kenko". Red de Salud Corea. La Paz, Bolivia.

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Corresponding author: Jhossmar Cristians Auza-Santivañez

ABSTRACT

Bioprocess engineering is undergoing an unprecedented transformation, driven by the growing demand for complex and bioactive biological products across diverse industries, from pharmaceuticals to food and energy. Recent advances have redefined the efficiency, scalability, and sustainability of biomanufacturing. Key elements of this evolution include the widespread adoption of single-use systems, the integration of automation and artificial intelligence (AI) for precise control and predictive optimization, and the fundamental shift toward continuous bioprocessing. These innovations not only reduce costs and production times but also improve product quality and consistency, enabling the manufacturing of personalized therapies and high-value compounds. Scaling strategies have diversified to include both scale-up and parallel expansion (scale-out), tailored to specific product needs. Together, these developments are laying the foundation for more agile, cost-effective, and environmentally responsible biomanufacturing, preparing the industry to address global challenges in health and sustainability.

Keywords: Bioprocesses; Cell Culture; Bioreactors; Engineering.

RESUMEN

La ingeniería de bioprocesos se encuentra en una fase de transformación sin precedentes, impulsada por la creciente demanda de productos biológicos complejos y bioactivos en diversas industrias, desde la

farmacéutica hasta la alimentaria y la energética. Los avances recientes han redefinido la eficiencia, la escalabilidad y la sostenibilidad de la biofabricación. Elementos clave de esta evolución incluyen la adopción generalizada de sistemas de un solo uso, la integración de la automatización y la inteligencia artificial (IA) para un control preciso y una optimización predictiva, y el cambio fundamental hacia el bioprocesamiento continuo. Estas innovaciones no solo reducen los costos y los tiempos de producción, sino que también mejoran la calidad y la consistencia del producto, permitiendo la fabricación de terapias personalizadas y compuestos de alto valor. Las estrategias de escalado se han diversificado para incluir tanto el aumento de volumen (scale-up) como la expansión en paralelo (scale-out), adaptándose a las necesidades específicas del producto. En conjunto, estos desarrollos están sentando las bases para una biofabricación más ágil, rentable y ambientalmente responsable, preparando la industria para abordar los desafíos globales en salud y sostenibilidad.

Palabras clave: Bioprocesos; Cultivo Celular; Bioreactores; Ingeniería.

INTRODUCTION

The field of bioprocess engineering is undergoing rapid transformation, driven by emerging trends and technologies that promise to revolutionize industries ranging from pharmaceuticals to food production.[1] At its core, bioprocess engineering is dedicated to developing and implementing processes that use living organisms or biological systems to produce valuable products. This discipline is central to industries such as pharmaceuticals, food, and biofuels and involves the construction of specialized systems, such as bioreactors, that facilitate biological processes.[2]

The industry is moving towards innovative, sustainable, and continuous operations driven by innovation, digitization, and a patient-centric orientation.[3] This evolution directly responds to the steady increase in demand for biologics, including vaccines, monoclonal antibodies, and cell and gene therapies. This increase in demand underscores the critical need for advanced bioprocessing solutions.[4] The shift from traditional batch processing methods to continuous, automated, and digitized bioprocessing represents a fundamental reconfiguration of biomanufacturing workflows. This transformation is not merely an incremental improvement but a paradigm shift driven by internal industry pressures, such as the need to reduce costs, increase efficiency, and improve quality, and by external market demands, particularly in personalized medicine.[5] Adopting these transformative technologies is essential to overcome the limitations of traditional methods, which often fail to meet increasing production demands, high costs, and inherent variability.

Importance and Diverse Applications of Bioprocess Engineering

Bioprocess engineering is a critically crucial multidisciplinary field with many industrial applications. This field ranges from the production of food and beverages, such as dairy products (cheese, yogurt), alcoholic drinks (beer, wine), and plant products (soy sauce, tofu), to food additives and supplements (flavors, proteins). In the health sector, it is essential to manufacture enzymes used in various industries (health, food, laundry, paper, and textiles), as well as to produce biopharmaceuticals, including vaccines, monoclonal antibodies, and cell and gene therapies.[2]

In addition, bioprocess engineering is vital in producing biofuels such as ethanol, biodiesel, butanol, biohydrogen, and biogas, using micro-organisms in bioreactors. In environmental applications, it contributes significantly to wastewater and solid waste treatment, soil bioremediation, and mineral recovery, taking advantage of the organisms' ability to use pollutants as a food source. Biofilters, for example, use microorganisms to remove pollutants and odors from the air. Modern bioprocess engineering harnesses genetic and molecular biology techniques to improve yields and product efficiency.[2] It is essential for developing transformed micro-organisms and new biocatalysts and understanding the interactions of proteins with their environments, allowing the production of new molecules or the optimization of existing bioproducts.[2]

The broad applicability of bioprocess engineering in sectors as diverse as health, food, energy, and the environment underlines its key role in building a sustainable, bio-based economy. This means the field goes beyond mere pharmaceutical production, encompassing solutions to global challenges such as climate change and healthcare needs. The ability to apply fundamental engineering principles (reactor design, cell culture, scale-up) to such varied outcomes indicates a powerful and versatile discipline. This versatility suggests that innovations in one sector, such as biopharmaceuticals, can potentially benefit others, such as food production or biofuels, fostering the need for interdisciplinary knowledge transfer and collaboration.[2]

DEVELOPMENT

New-generation cell culture technologies

The biotechnology market has experienced significant growth, driven by innovations such as single-use

technologies, advanced cell lines, and next-generation bioreactors.[9]

Single-use bioprocessing systems have marked a fundamental shift in recent years. These systems, which employ disposable components such as bags, tubing, and filters, offer substantial advantages over traditional stainless steel bioreactors, including reduced cross-contamination risk, greater production scale-up flexibility, and faster set-up times. The single-use bioprocessing market is experiencing exponential growth, with projections indicating an increase from $28,45 billion in 2023 to $128,24 billion by 2033. This growth reflects industry recognition of these systems' efficiency and cost-effectiveness, especially for small-scale or clinical-scale production where flexibility and speed are paramount.[4] It is estimated that single-use systems can save more than 60 % compared to fixed-asset stainless steel bioreactors.[5]

The integration of automation and digital technologies is revolutionizing bioprocessing. This advancement is driven by the industry's focus on improving efficiency, reducing human error, and enabling real-time monitoring and control of bioprocesses.[1] Automation is considered a critical area for the maturation and optimization of the biopharmaceutical industry, as it streamlines production processes, improves consistency, and ultimately reduces costs.[4] Robotic systems are employed for cell culture, media preparation, and sample handling tasks.[10]

Integrating artificial intelligence (AI) and machine learning (ML) improves the performance of bioprocessing systems, resulting in increased speed to market and improved product yields.[5] AI can analyze vast data sets to predict optimal bioreactor conditions, optimize process parameters, and automate tasks.[1]

The convergence of single-use systems with automation and AI creates a synergistic effect, facilitating a 'smart and flexible factory' model that significantly reduces risks and accelerates biopharmaceutical development and manufacturing, especially for personalized medicines. Single-use systems provide the physical flexibility and speed for diverse product portfolios and customized medicine. Automation and AI, meanwhile, provide the precision, control, and data intelligence to manage these flexible systems efficiently, consistently, and with minimal human error. Combining these technologies enables a modular and agile manufacturing environment that can quickly adapt to changing demands, reduce time to market, and ensure product quality, which is critical for novel therapies. This 'smart and flexible factory' model emerges as a direct consequence, addressing the core challenges of cost, speed, and quality in a dynamic biopharmaceutical landscape.[11,12]

High-Density Cell Culture and Seed Culture Intensification

High-density cell culture is highly desirable in industrial processes due to improved productivity, high volumetric productivity, reduced culture volume (downstream processing), facilitated cell separation, improved product recovery yields, and reduced costs.[13]

Seed culture intensification is a key approach in upstream bioprocessing to ensure efficient and flexible production processes for new drugs. It allows laboratories and manufacturers to grow adequate cell lines for inoculation of production bioreactors. High cell density cryopreservation (HCDC) is central to seed culture intensification, involving the aliquoting of high-density cell cultures into single-use bioprocessing containers and their cryopreservation. This method is time-saving and cost-effective, leading to faster cell growth, higher cell densities, and a reduction in the use of resources and facility space. HCDC ensures reproducibility of the process with optimal cell viability after thawing and maintains cell culture consistency and viable cell density (VCD), reducing batch-to-batch variability.[14]

The focus on high-density cell culture and seed culture intensification represents a strategic shift from simply growing more cells to growing cells more intelligently and efficiently earlier in the process. This upstream optimization has a positive cascading impact on downstream processing and overall manufacturing economics. Larger downstream bioreactors can be inoculated with a higher initial cell density by achieving higher cell densities upstream, significantly shortening the overall production run time and increasing throughput. This efficiency 'breakthrough' reduces the required bioreactor volume, media consumption, facility footprint, and associated operational costs throughout the bioprocessing chain. It also directly impacts downstream processing by providing a more concentrated feed, simplifying purification. This illustrates a critical cause-and-effect relationship: optimizing the earliest stages of cell growth positively multiplies the entire bio-manufacturing chain, leading to a more streamlined and cost-effective process.[15]

Perfusion Culture Systems: Techniques and Benefits

Perfusion culture is a continuous culture in which cells are retained or recycled back into the bioreactor, allowing for a constant supply of nutrients and waste removal to maintain a steady state.[16] This contrasts with fed-batch culture, where nutrients are added, but the medium is not removed.[14] The advantages of perfusion culture include a more efficient and cost-effective process.

The advantages of perfusion include a more sterile and stable culture environment, allowing long-term studies under the microscope.[17] It maintains high cell densities (50-100x106 cells/mL vs. 15-25x106 cells/mL for fed-batch) and achieves high volumetric productivity (0,5-2,0 g/L/day vs. 0,2-0,5 g/L/day for fed-batch). Compared to batch or fed-batch processes, this reduces workload and equipment footprint.[16]

Cell retention techniques for perfusion culture include filtration by centrifuge filters, tangential flow filtration (TFF), alternating tangential flow filtration (ATF), packed bed bioreactors with solid growth media such as Fibra-Cel® discs, and Cell-Lift impellers with decanter column.[16] Perfusion is crucial for 3D cell culture, as it mimics in vivo microenvironments and is valuable for drug research, cell biology, pharmacology, and regenerative medicine.[17]

The resurgence of perfusion culture, initially used for unstable products, is not only due to technological improvements but also represents a strategic response to biomanufacturing's economic and sustainability pressures. Its ability to maintain high cell densities and consistent product quality in a smaller footprint aligns perfectly with the general industry trend towards intensified and continuous processing, making it a cornerstone for future biomanufacturing. Historically, perfusion addressed low yields of unstable products. Modern biomanufacturing's economic and environmental demands are addressed with improved technologies (cell lines, media, retention devices). Its high volumetric productivity translates directly into a smaller facility footprint and reduced CAPEX, which is crucial for cost-sensitive biopharmaceuticals. In addition, its continuous nature aligns perfectly with the industry's shift towards continuous bioprocessing, enabling higher throughput and consistent product quality. This makes perfusion not just an operational choice but a key enabler for sustainable, cost-effective, and flexible biomanufacturing, especially as the industry moves towards diverse and personalized therapies.[18,19]

Innovations in Cell Line Engineering

The era of precision cell line engineering has arrived, leveraging CRISPR gene editing, targeted integration, and next-generation sequencing to sculpt cell lines with improved stability, productivity, and protein quality. Artificial intelligence and machine learning analyze vast datasets to predict optimal bioreactor conditions and anticipate bottlenecks.[11]

Multi-omics approaches (genomics, proteomics, metabolomics) comprehensively understand metabolic pathways and regulatory networks for targeted engineering, improving biomass yield, lipid content, and metabolite production. Cell-free technologies enable faster, more productive, and robust manufacturing processes with significantly reduced space and energy requirements, addressing the demand for plasmid DNA. Specific advances include predicting the stability of CHO cell lines using DNA repair gene expression, reconfiguring cell line development strategy to increase vaccine productivity, and enhancing CHO cell line development with a focus on product manufacturability through optimization and molecular engineering.[11]

Integrating advanced omics and AI with precision gene editing marks a shift from empirical cell line development to a highly rational, predictive, and accelerated engineering approach. This fundamentally alters the upstream bottleneck, enabling the creation of bespoke 'cell factories' that are more productive, robust, and predictable, directly impacting downstream efficiency and total cost of goods. Historically, cell line development could be a lengthy and somewhat empirical process. Current advances, particularly the fusion of precision gene editing (CRISPR), whole omics data, and predictive AI/ML, represent a move towards the rational design of cell factories. This means no longer just searching for the best natural variant but actively designing cells with specific desired traits (e.g., increased productivity, stability, specific glycosylation patterns, reduced impurity profiles). This rational design capability translates directly into:

- Accelerated development: faster identification and optimization of high-performing cell lines.
- Improved predictability and robustness: cell lines are designed to perform consistently under defined bioprocess conditions, reducing variability and risk during scale-up.
- Improved product quality: Direct engineering is needed for desired protein quality attributes.
- Reduced downstream burden: a cleaner and more consistent upstream product can simplify downstream purification.
- This fundamentally transforms the upstream bottleneck into a highly controlled and optimized starting point for biomanufacturing, impacting the entire value chain.

Innovative Approaches in Bioreactor Design

Modern bioreactors are intricate ecosystems designed to nurture and optimize cell growth. In them, the art of engineering converges with the science of biology to harness life for various applications.[20]

Regarding spatial configuration, designs incorporate baffles and novel geometries (e.g., the Clover Bioreactor's four-leaf clover shape) to promote uniform mixing and reduce dead zones, improving mass transfer and nutrient distribution.

Materials have significantly shifted from glass and stainless steel to single-use systems (disposable bags), which have revolutionized design by offering gentle mixing and oxygen transfer that are ideal for sensitive cell cultures (e.g., the Wave Bioreactor). Advanced materials include self-healing polymers, 3D printed scaffolds, smart glass coatings, and nanocomposite materials.[20]

Integrating advanced sensors enables real-time monitoring of critical parameters such as pH, dissolved

oxygen, and temperature. The IntelliCyt Bioreactor, for example, uses optical sensors to provide continuous data, enabling precise control. Integrated sensors provide continuous status checks, and advanced software interprets this data for on-the-fly adjustments. Software synergy couples the bioreactors with intelligent software (e.g., BioFlo 320) that can predict and adjust parameters for optimal growth conditions based on machine learning algorithms. Finally, the versatility of scale is a key feature, as modern bioreactors are designed to be scalable, from laboratory models to industrial giants (e.g., the Xcellerex XDR series), ensuring that processes developed on a small scale can be accurately replicated as scale increases.[20]

The evolution of bioreactor design goes beyond simple containment to create 'smart and adaptive microenvironments.' Integrating advanced materials, real-time sensing, and predictive software, this holistic design approach is crucial to handling novel biologics' increasing complexity and sensitivity and ensuring consistent performance at all scales. The collective goal of these design innovations is to transform the bioreactor from a static container to a dynamic, intelligent, and adaptive microenvironment that accurately mimics and optimizes conditions for biological processes. This is critical because modern biologics often involve more sensitive cell lines (e.g., mammalian cells for cell and gene therapies) and require particular growth conditions to ensure product quality and yield. The integration of advanced materials (e.g., single-use for sterility and flexibility), sophisticated geometries (for optimal mass transfer), real-time sensing (for continuous data), and AI-driven software (for predictive control) enables unprecedented accuracy and responsiveness. This holistic design approach mitigates traditional scale-up challenges (heterogeneity, shear stress) and supports the production of complex, high-value biological compounds with greater consistency and efficiency.[21]

Advanced Mixing and Aeration Technologies

Adequate mixing is critical to ensure the even distribution of nutrients, gases, and other essential components in the culture medium, which promotes consistent cell growth and maximizes productivity. In addition, it helps maintain uniform pH and temperature, preventing localized fluctuations that could negatively affect cell viability and process efficiency. Efficient mixing also favors the dispersion of gases such as oxygen and carbon dioxide in the liquid phase, ensuring optimal conditions for cellular respiration, gas exchange, and metabolic activities.[21]

Managing shear stress resulting from mixing is critical, as different cell types have varying sensitivities to turbulence. Appropriate mixing technology minimizes shear stress, preserving cell integrity and improving the robustness of the process.[21]

Stirred tank bioreactors (STRs) are widely used. They are characterized by a cylindrical vessel with a central stirrer that mixes the culture medium, ensuring uniform distribution and precise control of temperature, pH, dissolved oxygen, and nutrient availability. Diffusers introduce gases for efficient gas-liquid mass transfer. Although they offer precise control, STRs can generate high shear stress.[21]

Orbital shaking bioreactors (OSRs) use orbital shaking as an alternative mechanical agitation method instead of conventional impellers. This innovative technology achieves adequate mixing without stirrers, making it suitable for applications where gentle, shear-free mixing is essential.[21]

Wave bioreactors induce waves in sterile plastic bags for gentle agitation and bubble-free aeration, reducing shear stress and being suitable for smaller volumes and delicate cell lines.[21]

Other innovative techniques include spiral flow bioreactors (which introduce a helical flow to reduce dead zones), microbubble aeration (which provides a large surface area for gas exchange and minimizes shear stress), acoustic mixing (which uses sound waves for mixing without mechanical intrusion), and static mixers (geometric shapes within piping that guide fluids for efficient mixing and homogeneity).[20]

The diversification of mixing technologies beyond traditional impellers reflects a growing understanding of the nuanced needs of different cell types, especially sensitive mammalian cells. This specialization in blending is a direct response to bioproducts' increasing complexity and value, where maintaining cell viability and product quality outweighs the simplicity of a single blending approach. The variety of mixing technologies is not arbitrary; it is a direct consequence of the increasing diversity and sensitivity of biological systems used in bioprocessing, particularly for high-value products such as cell and gene therapies. Traditional STRs, while offering robust mixing and control, can impose damaging shear stress on mammalian cells and delicate stem cells. With their gentler agitation mechanisms, the emergence and adoption of OSRs and wave bioreactors signify a critical adaptation to preserve cell viability and, by extension, product quality and yield. This trend indicates a move towards tailored bioprocessing environments where the specific biological needs of the production organism dictate the engineered solution rather than a one-size-fits-all approach. This specialization is essential to maximize the production and quality of increasingly complex and sensitive biological compounds.[20,21,22]

Sustainability Considerations in Bioreactor Design

Sustainability has become a paramount concern in biomanufacturing, focusing on reducing operational carbon emissions, water consumption, and plastic waste generation.[3]

Bioreactor design incorporates sustainability through the following:

- Material efficiency: using recycled materials (e.g., 95 % recycled steel in the BioSteel Bioreactor) reduces ecological footprint and improves longevity and recyclability.[20] Manufacturers are exploring recycling options for single-use discarded components and using biodegradable polymers.[5]
- Energy optimization: innovative designs such as the HelixCore Bioreactor employ helical agitation systems that reduce energy consumption by 25 % compared to traditional impellers.[20]
- Waste minimization: Integrating on-site purification systems (e.g., the PurifyMax bioreactor with a built-in filtration module) recycles solvents and biomass, reducing waste by half. Waste reduction begins with the standardization of CIP systems through water-saving design and solvent collection methods.[20]
- Water conservation: designs like the AquaSaver Bioreactor include condensate recapture mechanisms that reuse 90 % of the evaporated water.[20]

The growing emphasis on sustainability in bioreactor design is not simply a corporate social responsibility initiative but an emerging economic imperative. By reducing resource consumption (energy, water, materials) and waste, sustainable design contributes directly to lower operating costs (OPEX) and potentially lower capital expenditure (CAPEX), aligning environmental management with business profitability. The drive toward sustainability in bioreactor design is increasingly intertwined with economic viability. While environmental regulations and corporate responsibility play a role, the tangible benefits of reduced energy, water, and material consumption translate directly into lower operating expenses (OPEX). In addition, innovations such as smaller footprints enabled by intensified processes (e.g., perfusion) can reduce capital expenditure (CAPEX) for new facilities. Therefore, sustainable design is evolving from a 'desirable' to a 'must-have' for competitive advantage, improving both environmental performance and the bottom line. This indicates a shift towards a more integrated approach where economic and environmental metrics are optimized in tandem.[20,22]

Challenges in Bioprocess Scale-up

Bioprocess scale-up presents numerous challenges, including maintaining product consistency, ensuring process efficiency, and complying with regulatory requirements.[23]

Heterogeneity is a significant problem; transitioning from laboratory scale to industrial production often leads to temperature, pH, and nutrient supply variability, affecting product quality and yield. Shear stress is another critical challenge; increased agitation and aeration in large bioreactors can damage delicate cells, reducing viability and productivity. This requires careful bioreactor design and optimization of mixing strategies. Oxygen transfer rate (OTR) can become a limiting factor in large bioreactors due to reduced surface-to-volume ratio, leading to anaerobic conditions. Efficient aeration systems, oxygen vectors, or microbubbles are needed to mitigate this problem.[23]

Consistency of feedstock is critical; variability in the quality and composition of starting materials can cause fluctuations in process yield and product quality, requiring strong supply chain management and stringent quality control measures. Regulatory compliance is rigorous in the biopharmaceutical industry, with strict regulations to ensure product safety, efficacy, and quality, involving extensive documentation, validation, and quality assurance processes. Economic considerations are also substantial, with costs associated with large-scale equipment, facility construction, and process validation. Finally, the environmental impact of large-scale bioprocesses cannot be overlooked; scale-up must minimize waste generation, energy consumption, and resource use, integrating sustainable practices.[23]

The challenges in scaling up bioprocesses center primarily on maintaining biological equivalence despite physical changes in scale. Engineers must compensate for non-linear mass/heat transfer changes and shear forces, which are often difficult to predict and model, underscoring the continued need for sophisticated computational tools and empirical validation. The main difficulty scaling bioprocesses stems from the non-linear relationship between bioreactor volume and critical physical parameters such as mass transfer (oxygen, nutrients, waste removal) and shear forces. While biochemical kinetics is scale-independent, the physical environment changes dramatically. Larger volumes inherently lead to reduced surface-to-volume ratios, which makes oxygen transfer and heat removal less efficient and requires more vigorous agitation, increasing shear stress on sensitive cells. This means that a direct 'doubling' of inputs does not guarantee a doubling of output or maintenance of product quality. Therefore, successful scale-up requires engineering adjustments and a thorough understanding of how these physical changes impact the system's biology, often requiring complex computational fluid dynamics (CFD) modeling and extensive empirical validation to maintain 'biological equivalence' at all scales. This highlights that scaling is a complex interaction of engineering physics and biological response, not a simple geometric extrapolation.[6,23]

Scaling Strategies (Scale-Up vs. Scale-Out)

Scalability is a fundamental challenge in bioprocessing, particularly in cell culture-based manufacturing.

Manufacturers choose between two main strategies to increase production capacity: scale-up and scale-out.[6]

Scale-up involves increasing batch size by transitioning to larger bioreactors, a common approach in producing industrial-scale biologics (monoclonal antibodies, vaccines). It requires extensive process optimization to maintain consistency of key parameters (oxygen transfer, nutrient distribution, pH control) at higher volumes. Challenges include homogeneous conditions, oxygen transfer limitations, shear forces, nutrient distribution, and complex regulatory validation. It is advantageous when a single high-volume batch is needed and regulatory approval for large-scale validation is feasible.[6]

Scale-out maintains smaller volumes but increases throughput by running multiple flasks or vessels in parallel. It is particularly relevant for personalized medicine (cell therapy), where small-batch, patient-specific therapies require highly controlled conditions. It commonly uses modular manufacturing environments with multiple small-scale single-use systems. Challenges include logistical complexities, increased labor demands, a larger facility footprint, and higher quality control costs. It benefits individualized batches, maintaining identical crop conditions, and flexibility in production scheduling.[6]

Te strategic choice between up-scaling and parallel scaling reflects a fundamental divergence in bio-manufacturing paradigms, driven by product type and market demand. Bottom-up scale-up optimizes economy of scale for blockbuster drugs, while parallel scale-up prioritizes flexibility and individualization for personalized therapies. This bifurcation requires different facility designs, automation strategies, and regulatory approaches, indicating a mature industry adapting to a diversified product portfolio.[6]

Critical Parameters for Successful Scale-up

It is desirable to replicate the performance of the optimized small-scale process on a larger scale, ideally without the need for much additional optimization on large workloads. This requires reproducing the growth environment of cells or organisms at all scales. The reaction volume is virtually unaffected by kinetics and thermodynamics, but mass transfer within a process is highly scale-dependent.[24]

Standard singular parameters to control include:

- Constant power input per liquid volume (P/V): this is probably the most widely applied strategy for scale-up, as mechanical shear stress, mixing quality, oxygen mass transfer, and carbon dioxide removal in aerobic cultures depend on the specific power input. Impellers with a higher power number (Np) can operate at lower speeds for adequate mixing, which minimizes the shear stress on the cells.[24]
- Constant tip speed: The tip speed of the impeller correlates with the fluid velocity at the impeller tip and is therefore related to the shear force on the cell or biological product. It also influences mixing time and oxygen transfer. Keeping the tip speed constant maintains a relatively constant level of shear force but may reduce mixing time performance in large-scale vessels. Increasing the tip speed of the impeller can improve mixing when scaling, but this can increase shear stress and should, therefore, be limited in cultures with higher sensitivity, such as mammalian cells.[24]
- Other key considerations for industrial scale-up include:
- Formulation adjustment: ingredients may behave differently at larger scales; unwieldy or hazardous ingredients become significant problems in bulk. The sourcing and cost of ingredients need to be re-evaluated.[25]
- Building codes: Understanding applicable building codes is crucial, as they dictate location and can impose significant costs due to hazard classifications.[25]
- Equipment selection: new or modified equipment is needed for larger batches, requiring a thorough analysis of capital/operating costs, size, footprint, operability, and cleanability.[25]
- Instrumentation and diagnostics: more data collection points are needed at larger scales for monitoring and troubleshooting. The decision between intermittent sampling or continuous monitoring determines the instrumentation needs.[25]
- Cleaning and sterilization need: methods differ significantly on a large scale and impact project cost. Inadequate cleaning can lead to poor performance or contamination.[25]
- Process optimization: accurately predicting how modification of each component will impact performance is crucial, avoiding trial and error. Process modeling and simulation are essential tools.[25]

Successful scale-up is not just about designing larger vessels but a holistic and predictive approach that integrates fundamental engineering principles (mass/heat transfer) with biological responses and regulatory/economic realities. The shift from trial and error to modeling and simulation (digital twins) is critical to reducing large-scale operations risks and accelerating market time. The central difficulty scaling up bioprocesses lies in the non-linear relationship between bioreactor volume and critical physical parameters. While biochemical kinetics are scale-independent, the physical environment changes dramatically. Larger volumes inherently lead to reduced surface-to-volume ratios, which makes oxygen transfer and heat removal less efficient and requires more vigorous agitation, increasing shear stress on sensitive cells. Therefore, the emphasis on 'constant

power input per liquid volume' and 'constant tip speed' is an engineering attempt to maintain critical physical conditions that directly impact cellular health and productivity.[26]

However, these engineering parameters alone are insufficient. The need to 'fine-tune the formula,' 'select the right equipment,' and 'optimize the process' through 'process modeling and simulation' points to a shift from reactive problem-solving to proactive, data-driven design. The explicit instruction to 'not rely on trial and error '[25] and the mention of 'digital twins '[26] reveal that the future of successful scale-up lies in predictive analytics and virtual experimentation. This reduces the risks of significant capital investments, accelerates development timelines, and ensures consistent product quality before committing to physical construction, making scaling up a more scientific and less empirical process.[27]

Process Intensification for Improved Production

Process intensification (PI) is a transformative approach aimed at improving the efficiency and productivity of bioprocesses by optimizing resource utilization, reducing production time, and increasing throughput.[28] It often involves drastic changes in equipment and/or process design, such as moving from batch to continuous processing or integrating new steps.[15]

Up to a tenfold increase in yield can be achieved for upstream process intensification using swinging motion, stirred tank, and perfusion-enabled bioreactors.[28]

- Perfusion cell culture maximizes facility utilization, improves process flexibility, and minimizes costs through cell retention devices and continuous media exchange to obtain high cell densities and viabilities over extended periods. Modern implementation is simplified through novel cell retention devices, single-use technologies, and sophisticated control logic.[15]
- N-1 perfusion, a form of seed culture intensification, focuses on intensifying cell growth in the N-1 seed culture passage before the production bioreactor (N). This achieves a faster and more robust process while maintaining an existing fed-batch production scheme, leading to a higher initial cell density in the production bioreactor and shortened run times. It can increase yields, save time, maintain a fed-batch process, and minimize the impact of validation.[15]
- Seed culture intensification aims to generate ultra-high density cell cultures at optimal points during cell expansion, using a robust cell retention device before inoculation.[14] It reduces time in the production reactor, increases the number of batches per year, shortens overall seed culture times, reduces plant footprint, increases flexibility, and reduces the cost of goods sold (COGs).[15]
- High productivity harvesting (HPH) is a novel application that allows sterile harvesting from a fed-batch bioreactor, preparing it for processing in a capture column without centrifugation or depth filtration. It achieves the exact product titer in half the time or doubles the product with no additional time.[15]
- XCell ATF® systems simplify and intensify upstream bioprocessing by providing high cell retention, used for N-1 perfusion, HPH, long-term perfusion, and seed culture intensification.[15]
- KrosFlo® TFDF® (tangential flow depth filtration) systems simplify and intensify upstream bioprocessing of viral vectors, combining tangential flow and depth filtration for high cell density and excellent product transfer.[15]

For downstream process intensification, processing times and costs are reduced with membrane and continuous chromatography, viral inactivation, SPTFF, and other techniques.[28]

The proliferation of specific intensification methods (N-1 perfusion, HPH, advanced filtration) indicates a granular optimization of each bioprocess step, going beyond the bioreactor to encompass the entire workflow. This reflects a mature industry's pursuit of marginal gains that, in aggregate, yield significant economic and operational advantages. The detailed enumeration of specific IP methods (N-1 perfusion, HPH, XCell ATF, KrosFlo TFDF) reveals that process intensification is not a single concept but a multi-faceted strategy that addresses specific bottlenecks throughout the bioprocess. N-1 perfusion and seed culture intensification optimize initial cell expansion, providing a high-density inoculum that shortens subsequent production runs. HPH and advanced filtration technologies streamline harvesting and purification, addressing traditional downstream processing bottlenecks. This granular, step-by-step optimization, enabled by specialized equipment, signifies a mature industry's quest to maximize volumetric productivity and reduce non-value-added steps (e.g., cleaning and manual transfers). The collective impact of these individual intensifications is a significant reduction in total manufacturing footprint, capital investment, and operating costs, demonstrating that continuous improvement at every stage is crucial for competitive advantage.[15,28]

Benefits of Intensified Bioprocessing

Process intensification significantly benefits small biopharmaceutical companies, contract development and manufacturing organizations (CDMOs), and large pharmaceutical companies.[28]

Business benefits include miniaturized plant size, reduced capital expenditure (CAPEX) and operating expenditure (OPEX), distributed manufacturing, and faster time from research to commercialization.[15] It can reduce drug costs to maintain competitiveness.[28]

Process benefits include higher cell densities, increased productivity, improved critical quality attributes (CQA) of the product, wider process conditions, and continuous processing.[15] It aims to increase productivity and improve efficiency.[28]

The environmental benefits manifest in reduced energy use, waste generation, reagent use, and a reduced landscape footprint.[15] This leads to more sustainable and cost-effective biopharmaceutical manufacturing.[28]

The multi-dimensional nature of the benefits of process intensification (business, process, environmental) illustrates a shift towards a 'holistic value optimization' mindset in bio-manufacturing. This means that efficiency gains are no longer solely about performance but encompass a broader spectrum of strategic advantages, including market responsiveness, sustainability, and financial performance. The comprehensive list of benefits for process intensification demonstrates that the industry's focus has broadened from simply 'producing more' to achieving 'holistic value optimization.' Beyond direct process improvements (higher cell densities, productivity), IP offers significant business advantages (reduced CAPEX/OPEX, faster time to market, distributed manufacturing) and critical environmental gains (reduced waste, energy, footprint). This indicates that bio-manufacturers increasingly view their operations through a strategic lens that balances economic profitability, operational efficiency, and environmental stewardship. The ability to achieve multiple benefits simultaneously makes IP a compelling strategy for long-term competitiveness and resilience in a rapidly evolving marketplace.[28]

The paradigm shift towards continuous bioprocessing

Continuous bioprocessing represents a paradigm shift from traditional batch processing methods, enabling uninterrupted production, reduced batch-to-batch variability, improved product consistency, and lower production costs. The continuous bioprocessing market is experiencing remarkable growth, with a compound annual growth rate (CAGR) of 22,4 %, projecting from $201 million in 2022 to $599 million by 2028. This approach is particularly evident in upstream processes, where many companies implement continuous perfusion systems.[1,4]

Advantages over batch/fed-batch systems include reduced cycle times, lower capital and operating costs, real-time monitoring and control, and improved product consistency.3 Compared to batch techniques, It can achieve up to 35 % cost savings for an annual production of 100-500 kg. Hybrid installations reach profitability 2-2,5 years sooner.[18]

To address the limitations of batch systems, it is recognized that traditional systems struggle to keep pace with increasing demands, and dynamic conditions can lead to an imbalance in nutrient supply and waste accumulation, affecting product quality. The low volumetric productivity of fed-batch manufacturing requires extensive facilities, which increases costs.[30]

There are types of continuous manufacturing: hybrid (semi-continuous) manufacturing combines batch and continuous processes within a single production workflow, often used in early-stage clinical manufacturing for rapid material generation. End-to-end manufacturing covers all steps from cell culture to purification of the final product, maximizing efficiency, yields and minimizing footprint and COGs.[30]

The accelerated transition to continuous bioprocessing is a strategic imperative driven by the limitations of batch processing to meet the growing demand for biologics efficiently and cost-effectively. This shift fundamentally alters facility design, supply chain logistics, and regulatory oversight, signaling a long-term industrial transformation towards highly integrated, agile, and cost-optimized manufacturing. The shift to continuous bio-manufacturing is not simply an operational improvement; it is a strategic response to the inherent limitations of batch processing in a market characterized by high demand, cost pressures, and increasing product complexity. The benefits - higher productivity, lower costs, greater consistency, and smaller footprint - collectively enable a more agile and economically viable manufacturing model. This change has profound knock-on effects: it requires new (modular, smaller) facility designs, drives the adoption of advanced automation and real-time monitoring (PAT), and requires regulators to adapt (ICH Q13). Ultimately, continuous bioprocessing facilitates a more responsive supply chain, enabling manufacturers to meet fluctuating market demands more efficiently and cost-effectively, thereby transforming the entire bio-manufacturing value chain.[30]

Industrial applications of bio-based and bioactive compound production

Bioprocess engineering is essential for the mass production of biofuels, food, biopolymers, industrial enzymes, and pharmaceuticals. It also applies to advanced biotechnology and water treatment processes.[8]

In the biopharmaceutical sector, it is used to produce vaccines, antibiotics, and new drugs, ensuring their safety, efficacy, and stability.[7] The growing demand for biological products (monoclonal antibodies, cell and gene therapies) drives the need for advanced solutions. Food and beverages cover the production of dairy products, alcoholic beverages, vegetable products (soy sauce, tofu), food additives (flavors, proteins), and

ingredients such as oligosaccharides. Fermentation improves bioactivity, nutrient availability, and flavor.[19]

For biofuels, bioprocess engineering facilitates the production of ethanol, biodiesel, butanol, biohydrogen, and biogas using microorganisms in bioreactors. Genetic modification (CRISPR/Cas9) and metabolic engineering improve the production of biofuels from agro-food waste and lignocellulosic materials. It plays a vital role in wastewater and solid waste treatment, soil bioremediation, and mineral recovery, using pollutant-consuming organisms in environmental applications. Biofilters, for example, remove pollutants from the air. Enzymes produced mainly from fungi and bacteria are used in the health, food, laundry, pulp and paper, and textile industries. Genetic manipulation allows the production of various enzymes. Finally, bioactive compounds from sources such as Chlorella vulgaris are gaining ground for their anti-inflammatory, antioxidant, and immunomodulatory properties in nutraceuticals and cosmeceuticals.[19]

Expanding the range of biological and bioactive compounds produced through bioprocessing, from therapeutic proteins to sustainable materials and food ingredients, signifies a fundamental shift towards a bio-based economy. This diversification requires highly adaptable and efficient bioprocessing platforms capable of handling varied product characteristics and regulatory requirements. The breadth and increasing complexity of biological and bioactive compounds produced through bioprocess engineering underline its role as a central enabling technology for a future bioeconomy. This diversification means that bioprocessing platforms must be increasingly flexible and versatile to adapt to different cell types, product characteristics, purity requirements, and regulatory pathways (e.g., food vs. pharmaceuticals). It also implies an increasing demand for interdisciplinary expertise, as engineers must understand the biological nuances of each product while applying universal engineering principles. The trend suggests that bioprocess engineering is not just about improving existing products but about unlocking entirely new classes of sustainable and health-promoting compounds, directly addressing global challenges.

Advances in bioprocesses for therapeutic protein production

Therapeutic proteins are molecules of biological origin used to prevent, treat, or cure diseases, often produced by recombinant DNA technology.[30,31]

Optimization strategies include PEGylation, albumin fusion, amino acid manipulation, and glycosylation strategies to optimize protein properties and functionality.[33] Fusion proteins combine therapeutic properties. Production methods use recombinant DNA technology for efficient production in various expression systems.33 Mammalian cell expression systems are standard and require aseptic fluid management to ensure sterility and integrity.[32] Aseptic fluid management to ensure sterility and integrity.

Aseptic fluid management is essential throughout the fluid pathway to prevent contamination and physical stress, especially with changes in volume size. Modular and closed processes are increasingly being adopted. Single-use technologies play a crucial role in optimizing production, including filling (e.g., RoSS.FILL), freezing (e.g., RoSS.pFTU for precise control, minimizing ice crystals), and storage (e.g., RoSS® Shell for protection, RoSS.ULTF for ultra-cold storage). These technologies eliminate time-consuming cleaning and sterilization processes, reduce the risk of contamination, and allow for rapid product changeovers.[33]

Advances in therapeutic protein production, particularly integrating single-use technologies with aseptic fluid management and cryopreservation, represent a strategic move towards end-to-end closed-systems manufacturing. This addresses the critical need for sterility, consistency, and flexibility for high-value, often patient-specific biologics, reducing the risk of the entire production lifecycle, from cell culture to delivery of the final product. The impact of AI in bioprocessing transcends simple automation by enabling a transition from reactive control to predictive and prescriptive optimization. By analyzing complex, multivariate data sets, AI can uncover hidden correlations, anticipate deviations before they occur (predictive maintenance), and recommend optimal real-time adjustments (dynamic control). This capability transforms process development from iterative experimentation to simulation and virtual optimization (digital twins), significantly speeding up timelines and reducing risk. The ultimate implication is the development of increasingly autonomous and self-optimizing bio-manufacturing facilities that can adapt to changing conditions, maintain consistent quality, and achieve unprecedented levels of efficiency with minimal human intervention.[30]

Optimizing the Synthesis of Bioactive Compounds (e.g., fermentation of Chlorella vulgaris)

Bioactive compounds play a key role in human health, with applications in nutraceuticals, biopharmaceuticals, biosurfactants, biostimulants, and cosmeceuticals. Multidisciplinary research integrating biochemistry, molecular biology, and bioprocess engineering is crucial. Engineering approaches have been employed to improve biological properties and yields.[32]

A notable case study is the fermentation of Chlorella vulgaris. This unicellular green microalgae is gaining significant attention due to its high protein content and abundance of bioactive compounds.[19]

- For strain improvement, genetic engineering (CRISPR-Cas9, chloroplast transformation), metabolic optimization (overexpression of key enzymes, redirection of metabolic fluxes), mutagenesis, and adaptive

evolution are used to improve metabolic pathways and productivity. Omics approaches provide a holistic understanding of targeted engineering.[19]

- Regarding fermentation techniques, advances in high-density fermentation strategies, adaptive strain evolution, and real-time monitoring systems have greatly improved efficiency, scalability, and sustainability. Both submerged and solid-state fermentation are used, along with mixed fermentation processes. The adaptability of C. vulgaris to diverse carbon sources in heterotrophic culture improves growth rates and biomass yields.[19]
- Bioreactor design and optimization focus on improving light penetration, gas exchange, and nutrient distribution for high-density cultures. Precise control of pH, temperature, nutrient supply, and aeration is essential.[19]
- Advanced monitoring technologies provide real-time data for dynamic adjustment of environmental conditions.[19]
- Downstream processing uses advanced extraction methods (ultrasound-assisted, microwave-assisted, supercritical fluid extraction), and process integration (biorefinery approach) maximizes the utility by generating multiple valuable products.[19]

Optimization of bioactive compound synthesis, exemplified by Chlorella vulgaris, demonstrates a highly integrated 'fusion' approach that combines advanced biological engineering (strain improvement, omics) with sophisticated bioprocess engineering (fermentation, bioreactor design, real-time monitoring, advanced downstream processing). This holistic strategy is essential to unlock the full potential of natural bioresources and achieve economically viable and sustainable production of various high-value compounds. The detailed approach to optimize the synthesis of bioactive compounds, as demonstrated with Chlorella vulgaris, exemplifies a sophisticated 'fusion' model. This is not a linear optimization but a highly iterative and integrated process where advances in upstream biological engineering (e.g., CRISPR-Cas9 for strain improvement, omics for understanding metabolic pathways) directly inform and are enabled by advances in bioprocess engineering (e.g., high-density fermentation, custom bioreactor design, real-time control). The goal is to create highly efficient 'microbial cell factories' to produce desired compounds at scale. The emphasis on 'efficiency, scalability and sustainability'[19] through this integrated approach highlights that maximizing the production of bioactive compounds requires a holistic systems-level engineering perspective, from gene to bioreactor to purification, to achieve commercial viability and environmental responsibility.

Future Directions and Emerging Technologies in Bioprocess Engineering

The bioprocessing industry and the bioproduction sector will continue its rapid transformation.[33] Future trends include:

- Hyper-personalisation: real-time manufacturing of patient-specific therapies.[33]
- AI-engineered biologics: accelerating drug discovery and the assessment of manufacturing capacity.[33]
- Cell-free bio-manufacturing: portable and on-demand systems for remote locations[33].
- Decentralised production: micro-factories close to the point of care for critical biologics.[33]
- Biologics 2.0: New modalities such as RNA-editing therapies, exosomes, and synthetic cells.[33]
- Sustainability: continued focus on reducing environmental impact, including green bioprocessing strategies.[33]
- New electrochemical reactors for efficient hydrogen production and capture/delivery.[33]
- Catalytic polymer processing and recycling: conversion of plastic waste into higher value products.[33]
- Nanodevices: for medical applications, e.g., to disrupt Alzheimer's plaques.[33]
- Synthetic biology: enabling the engineering of micro-organisms and cells with enhanced capabilities, creating customized bioprocesses for novel products.[33]
- Advanced analysis and modeling: computational tools and machine learning algorithms for complex datasets, predicting outcomes, and optimizing design.[33]

The future of bioprocess engineering is characterized by a radical decentralization and customization of manufacturing, driven by advanced biological engineering (synthetic biology, Biologics 2.0) and enabled by portable AI-driven systems. This suggests a shift from extensive, centralized facilities to agile, distributed manufacturing networks, fundamentally altering the biopharmaceutical supply chain and the accessibility of therapies. The emphasis on 'hyper-personalization' and 'decentralized production' indicates that bio-manufacturing is moving towards a model where products will be manufactured closer to the patient and in smaller, more targeted batches.[33]

CONCLUSIONS

Bioprocess engineering is at the forefront of innovation, driving a fundamental transformation in producing

biological and bioactive compounds. The industry has evolved from batch processes to continuous, highly integrated systems, enabling unprecedented efficiency, cost reduction, and improved product quality.

Adopting single-use systems, combined with automation and artificial intelligence, has created a 'smart and flexible factory' paradigm. This synergy speeds up set-up times, reduces contamination risks, and enables real-time monitoring and control, which is crucial for producing advanced and personalized therapies. High-density cell culture and seed culture intensification strategies demonstrate a strategic approach to optimize the early stages of the process, which has a positive multiplier effect on the efficiency and profitability of the entire value chain. The revitalization of perfusion cultivation underlines the industry's ability to re-evaluate and adapt existing technologies to meet modern demands for high productivity, smaller footprint, and sustainability.

In bioreactor design, the transition towards 'smart and adaptive microenvironments' reflects a deep understanding of the specific biological needs of production organisms. Diversification of mixing and aeration technologies, coupled with a strong emphasis on sustainability, demonstrates a commitment to optimizing performance and environmental and economic impact.

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AUTHOR CONTRIBUTION

Conceptualization: Freddy Ednildon Bautista-Vanegas, Pablo Carías, Luis Mariano Tecuatl Gómez, Rebeca Rocio Gomez Rosales, Themis Karim Ocampo Gamboa, Jhossmar Cristians Auza-Santivañez, Estefannie Eunice Valverde Fernández, Blas Apaza-Huanca, Ingrid Neysa Cabezas-Soliz, Jose Luis Diaz-Guerrero, Ruben Marco Antonio Soliz-Mendoza.

Data curation: Freddy Ednildon Bautista-Vanegas, Pablo Carías, Luis Mariano Tecuatl Gómez, Rebeca Rocio Gomez Rosales, Themis Karim Ocampo Gamboa, Jhossmar Cristians Auza-Santivañez, Estefannie Eunice Valverde Fernández, Blas Apaza-Huanca, Ingrid Neysa Cabezas-Soliz, Jose Luis Diaz-Guerrero, Ruben Marco Antonio Soliz-Mendoza.

Formal analysis: Freddy Ednildon Bautista-Vanegas, Pablo Carías, Luis Mariano Tecuatl Gómez, Rebeca Rocio Gomez Rosales, Themis Karim Ocampo Gamboa, Jhossmar Cristians Auza-Santivañez, Estefannie Eunice Valverde Fernández, Blas Apaza-Huanca, Ingrid Neysa Cabezas-Soliz, Jose Luis Diaz-Guerrero, Ruben Marco Antonio Soliz-Mendoza.

Research: Freddy Ednildon Bautista-Vanegas, Pablo Carías, Luis Mariano Tecuatl Gómez, Rebeca Rocio Gomez Rosales, Themis Karim Ocampo Gamboa, Jhossmar Cristians Auza-Santivañez, Estefannie Eunice Valverde Fernández, Blas Apaza-Huanca, Ingrid Neysa Cabezas-Soliz, Jose Luis Diaz-Guerrero, Ruben Marco Antonio Soliz-Mendoza.

Methodology: Freddy Ednildon Bautista-Vanegas, Pablo Carías, Luis Mariano Tecuatl Gómez, Rebeca Rocio Gomez Rosales, Themis Karim Ocampo Gamboa, Jhossmar Cristians Auza-Santivañez, Estefannie Eunice Valverde Fernández, Blas Apaza-Huanca, Ingrid Neysa Cabezas-Soliz, Jose Luis Diaz-Guerrero, Ruben Marco Antonio Soliz-Mendoza.

Software: Freddy Ednildon Bautista-Vanegas, Pablo Carías, Luis Mariano Tecuatl Gómez, Rebeca Rocio Gomez Rosales, Themis Karim Ocampo Gamboa, Jhossmar Cristians Auza-Santivañez, Estefannie Eunice Valverde Fernández, Blas Apaza-Huanca, Ingrid Neysa Cabezas-Soliz, Jose Luis Diaz-Guerrero, Ruben Marco Antonio Soliz-Mendoza.

Supervision: Freddy Ednildon Bautista-Vanegas, Pablo Carías, Luis Mariano Tecuatl Gómez, Rebeca Rocio Gomez Rosales, Themis Karim Ocampo Gamboa, Jhossmar Cristians Auza-Santivañez, Estefannie Eunice Valverde Fernández, Blas Apaza-Huanca, Ingrid Neysa Cabezas-Soliz, Jose Luis Diaz-Guerrero, Ruben Marco Antonio Soliz-Mendoza.

Writing – original draft: Freddy Ednildon Bautista-Vanegas, Pablo Carías, Luis Mariano Tecuatl Gómez, Rebeca Rocio Gomez Rosales, Themis Karim Ocampo Gamboa, Jhossmar Cristians Auza-Santivañez, Estefannie Eunice Valverde Fernández, Blas Apaza-Huanca, Ingrid Neysa Cabezas-Soliz, Jose Luis Diaz-Guerrero, Ruben Marco Antonio Soliz-Mendoza.

Writing – review and editing: Freddy Ednildon Bautista-Vanegas, Pablo Carías, Luis Mariano Tecuatl Gómez, Rebeca Rocio Gomez Rosales, Themis Karim Ocampo Gamboa, Jhossmar Cristians Auza-Santivañez, Estefannie Eunice Valverde Fernández, Blas Apaza-Huanca, Ingrid Neysa Cabezas-Soliz, Jose Luis Diaz-Guerrero, Ruben Marco Antonio Soliz-Mendoza.