

Review

Bioprocess Design and Optimization for Pharmaceutical Production Using Microalgae and Cyanobacteria

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Abstract

Microalgae and cyanobacteria have emerged as platforms for producing recombinant biologics, vaccine antigens, and bioactive compounds of pharmaceutical interest. However, their translation beyond proof-of-concept remains limited by light-field heterogeneity, gas-liquid mass-transfer constraints, product instability, and matrix complexity, all of which affect recovery, selectivity, and batch comparability. This review synthesizes and organizes published evidence using a process-engineering framework organized around product class, product localization, upstream–downstream coupling, and photobioreactor scale-up. It further considers the role of Quality by Design (QbD), model-informed development, techno-economic assessment (TEA), and life cycle assessment (LCA) in route selection and quality-oriented process development. Across the reported routes, the dominant burden shifts from disruption and clarification in intracellular products to extracellular stability and time-to-capture in secreted products, whereas biomass-based formulations are governed by potency and stabilization consistency, and analog-rich metabolites by profile control and selective fractionation. Current limitations include the scarcity of models that incorporate quality attributes as explicit outputs, the incomplete representation of regulated manufacturing burdens in TEA and LCA, and the lack of minimal, reproducible analytical panels adapted to product class and matrix. By framing these organisms as pharmaceutical process platforms rather than as hosts assessed only by titer, this review provides an engineering basis for scale-up, route prioritization, and controllable manufacturing.

Keywords: microalgae; cyanobacteria; Quality by Design (QbD); upstream–downstream coupling; photobioreactor scale-up; techno-economic assessment (TEA); life cycle assessment (LCA)



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1. Introduction

Pharmaceutical biomanufacturing has increasingly shifted from small molecules to biologics and advanced product formats, which depend not only on productivity but also on maintaining structural integrity, purity, potency, and batch consistency. As a result, process design can no longer be seen as a step that follows biological demonstration; instead, it must be developed from the beginning as the foundation for meeting specifications despite variability, formally managing risk, and maintaining performance throughout the product's lifecycle [1,2]. From this perspective, the key question is not just whether a host can produce

a target molecule, but whether cultivation and recovery can be turned into a controllable, repeatable, and verifiable manufacturing process that aligns with quality standards and accommodates planned process changes [3].

Within this framework, microalgae and cyanobacteria have gained attention as photo-synthetic platforms to produce recombinant proteins, vaccine antigens, and other bioactives of biomedical interest, because they combine carbon-fixing metabolism with an expanding repertoire of genetic engineering tools and, in some cases, the possibility of oral or biomass-based delivery [4–8]. These microorganisms are phylogenetically and physiologically diverse oxygenic phototrophs that convert light energy and inorganic carbon into biomass and a broad spectrum of metabolites, which has supported their exploration as sustainable hosts for high-value compounds [9–11]. Recent reviews indicate that this field has moved beyond isolated proof-of-concept reports, particularly for vaccine antigens, recombinant therapeutics, and biomass-associated delivery strategies, although downstream processing, product stability, and batch-to-batch consistency continue to represent major translational barriers [4–6,8]. In parallel, cyanobacteria remain attractive as programmable cell factories for therapeutic proteins and natural products, especially when chassis selection, pathway engineering, secretion behavior, and robustness under cultivation directly influence scalability and process feasibility [12,13]. This progression from proof-of-concept expression toward more application-oriented pipelines is also consistent with recent reviews focused specifically on recombinant and therapeutic proteins in microalgae and on microalgae-made human vaccines and therapeutics [7,8].

Nevertheless, the main bottleneck remains mainly engineering-driven. Even when a photosynthetic host produces a functional antigen or therapeutic molecule, moving from lab results into manufacturing is limited by light-field heterogeneity, CO₂/O₂ management, reactor-scale hydrodynamics, optical fouling, matrix complexity, product instability, and downstream incompatibilities that worsen as cultivation moves beyond flask-scale conditions [14,15]. In photobioreactors, self-shading, mixing-induced light–dark cycling, and spatial gradients in dissolved gases create heterogeneous physiological states that can influence biomass growth, active fraction, impurity levels, and recoverability, thereby making it difficult to directly compare small-scale results with larger-scale ones [14,16]. This aligns with broader analyses of the laboratory-to-field “yield gap,” which show that commercial adoption in the microalgae sector is limited more by controllability, operational stability, and scale-dependent operating constraints than by biological feasibility [15].

Accordingly, the pharmaceutical relevance of these organisms is determined not only by their ability to express a target molecule but also by whether cultivation and recovery can be translated into a process that maintains potency, purity, and comparability during scale-up and controlled change [1,2,7]. Under this stricter pharmaceutical framework, the process goal is not to maximize biomass or titer under average conditions, but to achieve a predefined, risk-justified quality profile that can be verified through measurement, release testing, and control of process-related impurities [1,2]. This requirement becomes especially challenging in phototrophic systems because upstream conditions affect not only how much product is produced but also where the product is located, which fractions co-partition with it, how stable it remains in the culture matrix, and how difficult it will be to clarify, capture, purify, and justify analytically under specifications [14,16]. Product localization is therefore not just a descriptive trait but a key design variable. An intracellular product generally increases disruption and clarification difficulties; a secreted product reduces solids but increases exposure to extracellular degradation and residence-time effects; and a biomass-based dosage form lowers purification requirements but shifts control toward potency, stabilization, and batch consistency [13,17]. In that sense, platform selection is not merely a biological choice but an engineering decision because it predefines product

localization, impurity burden, recovery route, and the set of CPPs needed to maintain CQA during manufacturing [1,3].

From this perspective, microalgae and cyanobacteria are not interchangeable as pharmaceutical hosts because their cellular structure, secretion behavior, co-extractives, and process sensitivities lead to distinct impurity profiles, recovery pathways, and analytical challenges that must be considered early in development [13,18]. This interdependence makes upstream–downstream integration a design requirement rather than just a methodological choice. In photosynthetic systems, choices about photobioreactor design, mixing, CO₂ supply, light exposure, and temperature influence biomass composition and the levels of potential impurities, which in turn affect the separation and purification processes needed to meet specifications [14]. When this coupling is not clearly addressed, the feasibility of different routes depends heavily on decisions about harvesting, concentration, drying, clarification, and purification, often leading to increased energy use and costs [19,20]. Recent studies on microalgae-based vaccine platforms also highlight that host selection, antigen localization, and delivery method significantly shape downstream strategies and practical feasibility [8].

To turn these couplings into a manageable pharmaceutical process, development must rely on tools that link product goals to process knowledge and route prioritization. Quality by Design (QbD) offers a systematic framework connecting target product profiles to critical quality attributes (CQAs), critical process parameters (CPPs), and the definition of the design space. Additionally, DoE, scale-aware modeling, and process-analytical approaches are essential for addressing the nonlinearities arising from light transfer, gas exchange, and culture optics [20,21]. In line with this, Bioprocessing 4.0 has been proposed as a practical framework for integrating analytics, modeling, data infrastructure, and decision support; however, its value lies in reducing control risk and enhancing operational decision-making rather than serving as a disconnected technological overlay [21]. Similarly, techno-economic assessment (TEA) and life cycle assessment (LCA) are crucial not merely as post hoc validations but as early decision-making tools to compare routes and identify real improvement opportunities within manufacturing constraints [19,20]. The recent bioprocessing literature beyond algae emphasizes this trend by positioning data integration, model-supported development, and smarter process monitoring as essential for robust biomanufacturing rather than optional features [21]. Accordingly, upstream operating conditions, product localization, co-extractive burden, and downstream sensitivity should be addressed as a single process-design challenge because each cultivation decision preconfigures the material entering recovery, thereby influencing the analytical and control requirements needed to ensure pharmaceutical comparability (Figure 1).

This review explores how platform choice, product localization, upstream conditions, downstream architecture, and QbD-focused design tools collectively influence the feasibility of pharmaceutical production with microalgae and cyanobacteria. It specifically structures the discussion around product-class-driven platform selection, how cultivation variability impacts recovery and impurity levels, the role of DoE and modeling in establishing controllable operating regions, and the use of TEA/LCA to prioritize process options without unsupported extrapolation. Its importance lies in reconceptualizing these organisms as integrated pharmaceutical process platforms, where success depends on controllability, comparability, and manufacturability during scale-up and changes.

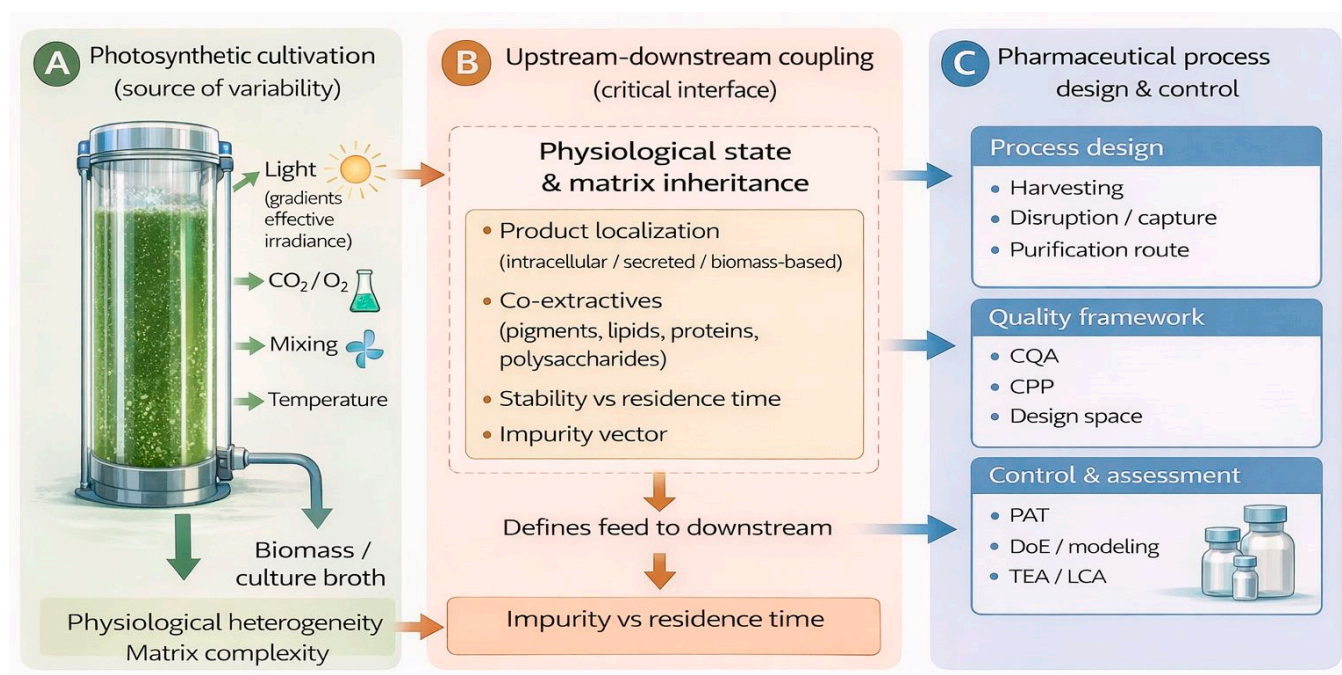


Figure 1. Upstream–downstream linkage and quality control (QbD) in photosynthetic bioprocesses.

2. Biological Platforms and Selection Criteria

2.1. Microalgae vs. Cyanobacteria

In pharmaceutical biomanufacturing, the decision between microalgae and cyanobacteria is made at the process train level, because product localization, matrix composition, and sensitivity to degradation initially determine whether development will focus on intracellular recovery, supernatant capture, or biomass conditioning. This, in turn, influences the burdens related to clarification, stabilization, selectivity, and analytical control [2]. Under these circumstances, the distinction between the two platforms becomes meaningful only when viewed in terms of the burdens placed on recovery, impurity control, and batch consistency, rather than as a purely biological or taxonomic difference.

In microalgae, the most well-documented pharmaceutical approach has focused on antigens and recombinant proteins, particularly in systems where vaccine-relevant domains or therapeutic proteins are produced in biologically active forms [22]. However, when examining these systems from a bioprocess perspective, the critical issue is not just expression but the form in which the product is recovered. In most cases reported, the target remains intracellular or is only partially secreted, which means product release is accompanied by pigments, lipids, polysaccharides, endogenous proteins, and other co-extractives. This complicates clarification and increases the effort needed to demonstrate purity and consistency [17]. Recent reviews concentrating on recombinant and therapeutic proteins in microalgae, as well as on microalgae-produced human vaccines and therapeutics, highlight that this field has advanced beyond isolated proof-of-concept reports. They also emphasize that downstream processing, product stability, and delivery format are crucial for translation [7,8]. Ultimately, the value of microalgae as pharmaceutical hosts depends on whether the active component can be recovered without the matrix burden overwhelming separation, polishing, and analytical verification.

This distinction becomes clearer in *Chlamydomonas reinhardtii*-based vaccine systems. In the case of the severe acute respiratory syndrome coronavirus 2 receptor-binding domain, the product was reported on an algal platform and shown to be immunogenic in mice, which supports the biological feasibility of the host while still raising process considerations

related to biomass handling, co-extractive control, and the extent of downstream purification required for the intended route [4]. When using the same host to produce a secreted recombinant Spike protein, the primary challenge shifts from lysing cells to maintaining the stability of the extracellular product, minimizing protease-mediated degradation in the culture medium, and reducing the time before capture [23]. This interpretation aligns with recent analyses of microalgae-made vaccines, where host selection, antigen localization, and delivery format are already treated as variables that define process feasibility rather than as secondary descriptors [8,24]. Consequently, the same organism can impose significantly different process requirements depending on whether the target is retained in biomass, recovered after cell breakage, or captured from the supernatant.

In cyanobacteria, the pharmaceutical proposition separates into two lines with distinct process implications. The first corresponds to biomass-compatible hosts such as *Arthrospira platensis*, where therapeutic proteins can be produced in edible matrices and incorporated into oral delivery strategies supported by stable expression, scalable cultivation, and post-cultivation conditioning [25]. Under this route, the process objective changes materially because the priority shifts away from maximizing purified yield toward biological potency, storage stability, and batch consistency in the conditioned biomass. The second line corresponds to cyanobacteria as sources of bioactive natural products, including peptides and polyketides of pharmacological relevance, where the main challenge is not only production but also the selective recovery and analytical discrimination of structurally related compounds [18,26]. Therefore, when cyanobacteria are considered as sources of therapeutic metabolites, the relevant criterion is not which strain produces more under nominal conditions, but which platform enables separation, identity verification, and robust purity despite analog-rich chemical profiles.

These differences become more significant when impurity architecture is explicitly considered. In microalgae, intracellular recovery often involves pigments, lipids, and co-extractable polysaccharides that complicate clarification, increase fouling, and add to the analytical burden related to purity and comparability [17]. In cyanobacteria, the impurity profile can also include cell-envelope-associated components with diverse biological activities, such as lipopolysaccharide-like fractions whose toxicological importance cannot be directly inferred from enterobacterial benchmarks and must be addressed through measurement and formal risk management rather than assumptions [27]. In this context, the platform not only alters the culture's composition but also shifts the development focus, determining whether downstream processes will primarily involve clarification and decolorization, pyrogenic risk assessment, or selective fractionation of complex metabolite mixtures.

The comparison is further affected by the physical controllability of the cultivation system. In both microalgae and cyanobacteria, scaling up introduces light gradients, gas-liquid mass transfer limitations, and biofouling, all of which affect reproducibility and the physiological state associated with the product form [14]. However, these issues do not impact all platforms or product types equally. In secreted production methods, cultivation heterogeneity becomes particularly important because it can affect extracellular product stability and extend the time during which the target remains in the culture supernatant before recovery [17,28]. When the product is intracellular or biomass-associated, culture heterogeneity becomes particularly critical because it influences cell composition, co-extraction load, and the consistency of the material entering disruption or conditioning. This reflects the broader laboratory-to-field yield gap in microalgae, where controllability, operational stability, and scale-dependent behavior remain significant barriers to industrial-scale production [15]. Therefore, choosing between microalgae and cyanobacteria should be done in conjunction with selecting the photobioreactor type and operational regime, since product

stability and the distribution of quality attributes may depend more on light-field and CO₂/O₂ control than on the average flask-level yield [14,15].

From a bioprocess design perspective, the reported cases are insightful because they show where variability first leads to a loss of product quality or process robustness after the material leaves cultivation. In the specific case of secreted recombinant Spike produced in *Ch. reinhardtii*, extracellular instability in the culture supernatant makes harvest timing and residence time before recovery important variables for maintaining product integrity [23]. For intracellular or partially intracellular products, variability spreads through disruption and clarification, so cell state at harvest, disruption intensity, solids loading, and clarification sequence are the most influential factors on the quality of the feed entering capture [17]. In biomass-based oral formats, the main challenge is the consistency of the conditioned biomass, making culture uniformity, stabilization conditions, and potency retention during storage critical operational factors [29–31]. In natural products rich in analogs, variability becomes critical when upstream drift broadens congener profiles and complicates separation, so the variables that affect biosynthetic stability directly influence downstream selectivity and analytical discrimination [18].

At this stage, the main impact of platform choice is in determining which recovery pathway and which source of variability will influence development more—whether through biomass conditioning, extracellular instability, intracellular clarification, or selective fractionation of structurally related compounds. Table 1 shows the specific context where these challenges arise, while the next section discusses the upstream operating variables that make these challenges controllable during process development (Table 1).

Table 1. Reported cases of pharmaceutical biomanufacturing in microalgae and cyanobacteria and their impact on the process train.

Organism	Reported Pharmaceutical Product	Upstream Implication	Downstream Implication	Reference
<i>Arthrospira platensis</i>	Oral therapeutic single-domain antibodies (sdAbs)	Biomass scalability and consistency	Control of biological potency without chromatographic purification	[25]
<i>Chlamydomonas reinhardtii</i>	SARS-CoV-2 RBD (vaccine)	Culture-phase control for conformational stability	Oral biomass or partial purification; control of co-extractives	[22]
	Secreted recombinant Spike	Medium stability and reduced proteolysis	Capture from supernatant; structural heterogeneity	[23]
<i>Chlorella vulgaris</i>	VHSV vaccine glycoprotein	Genetic stability under induction	Whole matrix as an oral vehicle	[32]
<i>Nannochloropsis oceanica</i>	VP2 vaccine subunit	Variability among transformants; expression control	Intracellular recovery; intensive clarification	[33]
<i>Phaeodactylum tricornutum</i>	Monoclonal antibodies	Sensitivity to culture conditions that induce proteases	Degradation in the medium; need for early stabilization	[34]
Various species	Bioactive peptides/polyketides	Optimization of biosynthetic titers	Multistage separation due to structural analogs	[18]

2.2. Upstream Design and Control of Quality-Oriented Operating Conditions

Upstream design in photosynthetic platforms for pharmaceutical production should be seen as the stage at which the physiological state of the culture is maintained within a range that ensures product function, matrix manageability, and batch reproducibility. This is because the cellular response to light, CO₂/O₂ balance, and mixing not only influences the

amount of material produced but also affects the target's active fraction, its stability during cultivation, and the composition of the feed that will later undergo recovery. This sensitivity becomes more pronounced during scale-up since gradients of irradiance, inorganic carbon, and dissolved oxygen create subpopulations exposed to different light histories and gas-transfer conditions. These variations can lead to photo-oxidative stress, protease activation, metabolic redistribution, and changes in recoverability [14]. Under these conditions, upstream optimization cannot be limited to maximizing biomass or nominal titer, because a higher volumetric output might still yield lower usable yield if product integrity declines or the culture matrix becomes less compatible with downstream processing.

Within this framework, photobioreactor architecture and operating mode are no longer just about convenience but become critical quality-determining choices, because they define the optical path, light–dark cycling, gas transfer capacity, heat removal, and the culture's effective exposure history. Flat-panel systems favor shorter optical paths and more uniform irradiance at similar cell densities, thereby reducing localized photoinhibition and making thermal and pH control easier. These features are especially important when the key metric is the active fraction of a biologic rather than total biomass [14]. Tubular systems provide a larger illuminated area per unit volume and greater potential for scale, although they also make oxygen accumulation and carbon limitation more difficult to control when degassing and light distribution are not resolved along the flow path [15]. In column and airlift configurations, the practical advantage lies in simpler hydrodynamics and efficient gas–liquid transfer within defined operating ranges, although optical control is usually less direct, and the balance among light distribution, oxygen stripping, and carbon supply becomes more dependent on aeration and circulation conditions. Recent analyses of photobioreactor configurations and comparative hydrodynamic studies reinforce this interpretation by treating optical path, hydrodynamics, and gas-transfer capacity together during scale-up, particularly when oxygen removal and CO₂ transfer, often represented operationally through mass-transfer descriptors such as kLa, begin to constrain culture stability and performance [35,36].

The relevance of these variables becomes clearer when recombinant products are examined in terms of the burden they impose on the process. In *C. reinhardtii*, a functional severe acute respiratory syndrome coronavirus 2 receptor-binding domain has been reported at approximately 31 µg·g^{−1} wet biomass, and the productive context indicates that light regime, temperature, and culture condition at harvest affect not only expression but also the fraction that remains functionally useful for recovery [22]. Irradiance, therefore, cannot be treated as a simple growth lever, because light intensification may increase biomass while reducing the active fraction or altering matrix composition. In the case of a secreted recombinant Spike in the same host, the relevant upstream problem differs. When the recombinant product is secreted into the culture medium, the recoverable titer depends not only on secretion efficiency but also on its extracellular stability before harvest and capture [23,28]. The operating objective then shifts from generating more product in culture to preserving integrity before capture, which is why harvest timing and early clarification enter the upstream control logic rather than remaining purely downstream adjustments.

A similar shift is observed when intensification strategies are introduced. Fed-batch heterotrophic or mixotrophic operation can raise biomass concentration and increase the concentration of secreted proteins, as shown in *C. reinhardtii* producing human intercellular adhesion molecule 1, where biomass values up to 40 g·L^{−1} and product concentrations near 50 mg·L^{−1} were reported [37]. Yet this gain in titer also increases sensitivity to feeding regime, pH control, and product stability in the supernatant, so the relevant question becomes whether intensification preserves a usable product rather than merely increasing concentration. In marine microalgae, the culture phase can be equally decisive.

In *Nannochloropsis oceanica*, a VP2 viral antigen was reported at up to 4.4% of total soluble protein, and the study indicates that physiological stage conditions the expression outcome, which means that harvest timing must be defined against culture phase if reproducibility is to be preserved [33]. In *Phaeodactylum tricornutum*, producing monoclonal antibodies, the critical issue is not only secretion but also the preservation of product integrity during cultivation, because medium-associated degradation has been reported, and therefore culture duration and medium condition become part of the upstream decision space rather than variables that can be corrected later by purification [34].

In cyanobacteria, the operating objective changes when biomass itself becomes the dosage matrix. In *A. platensis*, biomass-associated therapeutic proteins have been reported in dry-biomass formats, and constitutive expression of a therapeutic VHH fusion at 3% of dry biomass, equivalent to 8% of soluble protein, has been described under controlled cultivation conditions [25]. Under this route, the critical metric is not volumetric concentration of purified material, but the reproducibility of potency per unit biomass after cultivation and stabilization. This makes light intensity, temperature, CO₂ supply, and pH-controlled cultivation operationally relevant because they condition biomass consistency before drying and oral delivery. The same logic is reflected in phycocyanin production, where productivities around 123 mg·L⁻¹·day⁻¹ have been reported under defined light and cell-density conditions, indicating that effective irradiance and culture density influence not only yield but also the consistency of the product-bearing biomass [38]. In practice, this means that light control must be interpreted physiologically rather than nominally, because the same incident irradiance can produce different outcomes depending on optical path, cell concentration, and the resulting exposure history of the culture.

For metabolite-oriented routes, upstream control becomes even more tightly linked to downstream feasibility because the issue is not only how much product is formed but also which chemical profile is generated. In cyanobacterial natural products, operational variations can alter the relative abundance of structural congeners, thereby broadening the burden later imposed on selective fractionation and analytical discrimination [18,26]. In fucoxanthin-producing diatoms, pilot-scale productivities close to 2.5 mg·L⁻¹·day⁻¹ have been reported, and nutrient continuity together with stable cultivation has been identified as necessary to sustain metabolite-specific productivity and avoid physiological transitions that lower usable output [39]. Under these conditions, upstream design is not only a matter of productivity enhancement but also of profile restriction and route stabilization, because a chemically unstable feed propagates directly into higher separation cost, increased characterization burden, and weaker batch-to-batch comparability.

On this basis, the upstream operating problem changes with the product route. In intracellular products, effective irradiance, harvest state, and clarification-compatible biomass condition determine whether titer remains associated with a recoverable active fraction. In secreted products, medium stability, pH, temperature, and residence time in the supernatant delimit how much product can accumulate before degradation begins to dominate. In biomass-based dosage formats, culture uniformity, stabilization conditions, and potency retention during storage define whether the final material remains pharmaceutically usable. In analog-rich metabolites, the relevant upstream objective is not the widest possible production profile but a sufficiently constrained biosynthetic state to keep downstream selectivity tractable. The cases retained in Table 2 preserve the operating context in which these variables become decisive, allowing product class, reported yield, and upstream control burden to be read together before those burdens are translated into downstream requirements and formal control logic.

Table 2. Operational determinants and metrics for control of the photosynthetic upstream.

Platform	Organism	Product	Reported Titer/Yield	Critical Process Parameters	Operational Advantage	Reference
Microalga	<i>Chlamydomonas reinhardtii</i>	SARS-CoV-2 RBD	31 $\mu\text{g}\cdot\text{g}^{-1}$ wet biomass	Light regime, temperature.	Production of functional RBD with ER/secretory	[22]
		Secreted Spike	Transient accumulation ($\mu\text{g}\cdot\text{L}^{-1}$)	pH, temperature, residence time in medium	Lower solids burden	[23]
		Human ICAM-1	50 $\text{mg}\cdot\text{L}^{-1}$ up to 40 $\text{g}\cdot\text{L}^{-1}$ of biomass	Feeding (mixotrophy/heterotrophy), pH, stability in medium	Titer intensification	[37]
	<i>Nannochloropsis oceanica</i>	Viral VP2 antigen	Up to 4.4% of the total soluble protein	Culture phase, light, CO_2/O_2	High relative content	[33]
Diatom	<i>Phaeodactylum tricornutum</i>	Monoclonal antibody	1–2 $\text{mg}\cdot\text{L}^{-1}$	Light, oxidative-stress control	Secretion facilitates capture	[34]
		Fucoxanthin	2.5 $\text{mg}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$	Light, nutrients, culture stability	Evidence of pilot-scale scale-up	[39]
Cyanobacteria	<i>Arthrospira platensis</i>	Oral therapeutic sdAb	0.5–2 $\text{mg}\cdot\text{g}^{-1}$ dry biomass	Light, density, and inter-batch repeatability	Bioencapsulation reduces purification	[25]
		Phycocyanin	123 $\text{mg}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$	Effective light, cell density	Controllable continuous production	[38]
	Various species	Natural metabolites	Regime-dependent	Light, inorganic carbon, pathway stability	Upstream profile modulation	[18]

3. Pharmaceutical Downstream: Harvesting, Extraction, Purification, and Quality Assurance

In photosynthetic platforms, downstream does not behave as a black box that follows cultivation, because product localization, feed composition, and the type of co-mobilized impurities are determined by physiological state and by the harvesting and conditioning approach, and this inheritance conditions effective recovery and the ability to sustain critical quality attributes under controlled variability. In microalgae and cyanobacteria, the matrix includes chromophores (chlorophylls and carotenoids), amphiphilic fractions, and biopolymers (lipids, polysaccharides, endogenous proteins, and nucleic acids) that alter extract rheology, promote emulsions, and increase fouling of membranes and resins, with direct impacts on dynamic capacity, differential pressure, and operational reproducibility, such that the downstream train must be designed for selectivity and impurity control rather than for isolated volumetric efficiency [17]. This reality makes it insufficient to compare unit operations in the abstract, because performance depends on coupling between units; therefore, it is advisable to structure downstream as a decision architecture that responds to the dominant mechanism of product loss or quality degradation, avoiding unadjusted extrapolation of schemes inherited from heterotrophic processes where the co-extractive profile differs.

Operationally, this architecture becomes clear when three recurrent routes defined by product localization are formalized, since each imposes a different balance between recovery, variability risk, and analytical burden. When the product is predominantly intra-

cellular, the typical sequence is harvesting, biomass concentration, disruption, clarification, capture, and polishing, and the limiting step is often the generation of an amphiphilic colloidal extract, in which pigments and lipids compromise clarification and degrade capture performance [17]. When the target protein is secreted into the medium, downstream priorities shift from cell-disruption operations to supernatant clarification, capture from a dilute extracellular stream, and maintaining product integrity during the period before purification [23,40]. When the pharmaceutical product is pursued as a biomass-based formulation, the route concentrates on harvesting, washing, stabilization/drying, and potency control, reducing or eliminating chromatography, while shifting risk toward stability during conditioning, inter-batch consistency, and microbiological control, as has been demonstrated for biologics expressed in *A. platensis* intended for oral administration [25]. Within this framework, downstream becomes a quantifiable and operationally tractable problem insofar as each route enables identification of where losses of the active fraction originate and where variability is amplified, thereby guiding equipment selection and ordering.

Harvesting, as the first downstream interface, is often the point at which the type of feed that will enter disruption or capture is defined; therefore, technology selection must be grounded in principles and in characteristic failure mechanisms of algal matrices. Centrifugation exploits density differences and accelerates separation at high throughput, making it attractive for biomass recovery; however, its specific cost and effective shear increase when fine solids and viscous extracts appear, and mechanical stress can induce partial release of cellular contents, increasing soluble macromolecules and colloidal particles that subsequently blind membranes and resins and raise the demand for secondary clarification [17]. Tangential-flow filtration, in its microfiltration/ultrafiltration variants, is based on transmembrane pressure and tangential-flow control to limit cake formation and concentration polarization; nevertheless, in microalgae, fouling is often dominated by extracellular polysaccharides, lipids, and colloidal material that form compressible layers, such that practical design depends on effective pre-clarification, operating conditions that avoid cake compression, and considering diafiltration as a tool to decouple interferents rather than solely as a concentration step [17]. Flocculation, including electrochemical variants, can reduce energy consumption and simplify separation, but introduces an additional impurity vector through residual flocculants and salts that condition the rest of the train, which often translates into greater ion-exchange or diafiltration requirements to bring the feed to conditions compatible with capture and, above all, with impurity limits for the final product.

Clarification functions as an intermediate stage because it determines whether the process will be governed by efficient chromatographic capture logic or by a succession of plugging events, fouling-driven losses, and operational variability. This hinge is particularly critical in intracellular processes, where disruption and release generate amphiphilic colloidal suspensions. In algal extracts, particle size, effective surface charge, viscosity, and lipid fraction determine whether clarification can be achieved by secondary centrifugation, microfiltration, or staged schemes. The available evidence shows that a design combining controlled-intensity disruption with gradual clarification is often more robust than pursuing maximal release followed by aggressive cleaning, because lipid emulsions and fines generated by intense disruption increase pressure and reduce capacity in membrane steps and in columns [17]. Therefore, when the product is intracellular and the extract tends to be lipid-rich or pigmented, the pragmatic criterion is not to disrupt more, but to disrupt sufficiently to release the product while minimizing formation of a colloidal phase that sequesters product and degrades capture performance, whereas, in secretory routes, the

priority shifts toward early, low-shear microfiltration/clarification to minimize dead time and reduce aggregation or degradation events induced by supernatant handling.

For intracellular products, cell disruption concentrates equipment decisions with direct consequences for quality and cost. Bead milling and high-pressure homogenization provide effective release, but increase interfacial area and shear energy, promoting lipid emulsification, massive chromophore release, and the generation of submicroparticles that amplify fouling and, in biologics, can promote aggregation through exposure to interfaces and increased cellular fragments that act as nucleators. This interaction becomes visible when upstream conditions have already stressed the biologic, because intense disruption not only releases product, but also exacerbates heterogeneity and fragmentation; in monoclonal-antibody-producing diatoms, mAb degradation associated with cultivation stress has been described, and this antecedent requires downstream to select disruption and clarification with an integrity-preservation focus, since fragments and aggregates increase polishing burden and reduce overall yield even when apparent titer is favorable [34]. Osmotic or enzymatic alternatives can improve selectivity in specific cases, although their implementation at scale under cleaning, validation, and control schemes typical of regulated manufacturing tends to be more restrictive; therefore, in bioprocess design terms, robustness is often achieved by combining moderate mechanical disruption with clarification and capture designed to tolerate an inevitably complex matrix.

When the product is secreted, downstream changes occur without becoming trivial: the solids burden is reduced, but extracellular stability and timely capture become problems. In *Ch. reinhardtii*, secretion of recombinant Spike showed transient accumulation and proteolysis-associated degradation, which makes product residence time in the supernatant a variable that controls the active fraction available for capture; consequently, downstream design must synchronize clarification and direct capture with narrow operating windows and pH and temperature conditions that minimize degradation, because delays translate into real losses of functional product and increases in degraded variants that raise polishing effort [23]. Under intensified regimes, such as cultures in which human ICAM-1 production has been reported, increased supernatant concentrations can coexist with greater product sensitivity to medium conditions and processing times, reinforcing that the secretory train must be designed for rapid clarification and capture, while also incorporating membrane concentration and variant-oriented polishing, without assuming that the absence of disruption eliminates the purity and consistency challenge [37]. In diatoms, antibody secretion facilitates capture from the supernatant, although partial mAb degradation during cultivation necessitates additional polishing to remove fragments and aggregates; therefore, upstream and downstream cannot be assessed separately, because the reported titer loses meaning if the product reaches the capture train with compromised integrity [34].

During capture and purification, the decisive difference relative to classical processes is that pigments and amphiphiles occupy sites, increase backpressure, and degrade chromatographic performance. Choosing between selective early capture and prior cleanup is a structural decision. If the matrix is relatively controlled, early affinity capture or selective mechanisms can concentrate product and shift impurities to the flow-through; however, in extracts with a high lipid and pigmented fraction this strategy can fail due to fouling and capacity loss, forcing clarification, fractionation, or decolorization pretreatments that reduce chromophore load before columns, with the associated costs in solvents, consumables, and operational complexity [17]. In process design, capture and polishing must be connected because the impurity profile entering capture affects how much downstream refinement is needed to maintain product quality and analytical clarity. In secreted systems, fewer solids may not prevent extracellular degradation or product variability before filtration [41].

The impurity vector in these platforms translates biology into process decisions and intermediate assays. For example, in microalgae, pigments, lipids, and polysaccharides dominate clarification and capture variability, and affect absorbance- or fluorescence-based measurements and functional assays if not controlled. In contrast, endogenous proteins and nucleic acids contribute to the total load and co-purification risk, underscoring that the hinge clarification step not only improves operations but also defines the feasibility of reproducible purification [17]. In cyanobacteria, beyond chromophores and biopolymers, secondary metabolites and, in certain Gram-negative species, hazards associated with cell-wall components with toxicological implications appear, which requires incorporating a formal risk-management approach that links specific hazards to process controls and to appropriate assays without yet shifting the discussion into good manufacturing practice (GMP) [2]. This translation from matrix to control materializes in practice through intermediate sampling points that distinguish losses due to degradation, losses due to inefficient capture, and the emergence of variants or impurities during unit operations, because in algal matrices a substantial fraction of variability originates in disruption–clarification–capture transitions or clarification–concentration–polishing transitions, and not only in the final product.

Upstream titer sets the theoretical maximum; the released fraction depends on disruption or effective secretion, the captured fraction depends on selectivity and feed condition, the polished fraction depends on the need to remove variants and impurities, and the stable fraction depends on chemical or proteolytic degradation during residence times and during concentration and intermediate storage operations. In this secretory route, product loss can occur before capture if the recombinant protein remains in the supernatant for extended periods under conditions that promote breakdown, making timely recovery a crucial factor in preserving the product quality [23]; in intracellular routes, dominant loss is often associated with co-extraction, emulsions, and fouling that sequester product and reduce recoveries in clarification/capture, reinforcing that disruption and clarification must be designed as an inseparable pair [17]; in biomass-based formulations, dominant risk shifts toward potency as a function of moisture and stabilization conditions, making drying and conditioning determinant operations for the batch's pharmaceutical performance [25].

Finally, when the objective is cyanobacterial natural products of pharmacological interest, downstream is dominated by selectivity and congener control, because a mixture of analogs with similar polarity and mass makes separation inherently multistage and requires a purification strategy designed in concert with the analytical strategy to demonstrate identity and purity robustly. In this area, recent evidence underscores that the bottleneck is not only extracting more, but restricting profiles and designing fractionations that resolve closely related chemical families, because even with appreciable production, congener complexity increases cost and uncertainty in the downstream train and increases variability risk if the feed changes between batches [18]. This interaction between chemical profile and separability makes explicit that pharmaceutical downstream in photosynthetic systems is sustained when separation architecture is defined by dominant loss mechanisms and by the impurity vector accompanying the product. That definition enables, in the next section, the conversion of process decisions into an executable framework for design, control, and risk management in line with quality-by-design approaches.

In a photosynthetic pharmaceutical downstream, equipment selection is not justified by separation efficiency in the abstract, but by how each operation transforms the matrix and shifts risk across four domains that then govern performance and the demonstrability of quality: generation of colloids/emulsions that deteriorate clarification and capture, degradation and microheterogeneity of the biologic due to residence times and process stress, occupation/fouling of surfaces (membranes and resins) that reduces capacity and

reproducibility, and persistence of specific impurities that interfere with assays and product attributes. Under a risk-management approach, a robust decision does not ask which operation to use, but at which point in the train it is preferable to assume the cost of control to minimize propagation of variability and avoid later stages being dominated by fouling or by degraded species that are difficult to remove [2]. Under this criterion, downstream design can be formulated as an explicit mapping between product situation and feed type, the dominant operation and limiting loss mechanism, such that each equipment decision is linked to a verifiable control lever and to sampling points that allow isolating degradation, colloidal sequestration, or fouling before variability propagates into capture and polishing [2]. (Table 3).

Table 3. Downstream-limiting mechanism-design levers map in photosynthetic pharmaceutical biomanufacturing.

Route/Product Situation	Dominant Operation and Equipment	Limiting Mechanism in Algal/Cyanobacterial Matrices	Affected Attribute (Quality/Operation)	Downstream Design Lever	Reference
Intracellular product (biologic)	Harvesting by centrifugation	Increased effective shear with fine solids and non-Newtonian rheology; partial release of solubles/colloids carried into the extract	Subsequent clarification, soluble load, and reproducibility	Operating intensity and conditions that minimize premature release, coupled with staged clarification	[17]
	Concentration/clarification by tangential-flow filtration	Concentration polarization and formation of a compressible cake dominated by polysaccharides/lipids; flux decline and fouling-driven variability	Operating capacity, differential pressure, consistency	Effective pre-clarification, an operating regime that limits cake compression, and the use of diafiltration to decouple interferents	
	Disruption by bead milling	Lipid emulsification and generation of submicroparticles that degrade clarification and increase interfaces (aggregation risk)	Biologic integrity/aggregation; fouling	Minimum sufficient energy and a disruption → clarification sequence designed to avoid strongly emulsified extracts	
	Disruption by high-pressure homogenization	Increased interfaces and fine species; if the biologic arrives stressed, disruption amplifies fragmentation/heterogeneity	Fragments/aggregates; polishing burden	Select disruption intensity for integrity preservation rather than maximum release; reduce post-disruption dead times	[34]
Secreted product (biologic)	Early supernatant clarification	Extracellular degradation (proteolysis) and residence-time losses in the medium before capture; handling sensitivity	Potency and integrity; variant profile	Minimize time-to-capture; low-shear clarification and operation coupled to harvesting windows	[23]
	Direct capture + concentration by ultrafiltration/diafiltration	Concentration can exacerbate aggregation or retain interferents if the supernatant is insufficiently clarified	Aggregation; analytical interferences; purity	Define the clarification → capture/concentration order to limit interferent accumulation; control conditions that preserve conformation	
	Supernatant processing under intensified regimes	A higher titer can coexist with greater product sensitivity to medium conditions and process times	Product consistency; variability	Design for rapid processing under conditions that reduce degradation; avoid prolonged accumulation in the supernatant	[37]

Table 3. *Cont.*

Route/Product Situation	Dominant Operation and Equipment	Limiting Mechanism in Algal/Cyanobacterial Matrices	Affected Attribute (Quality/Operation)	Downstream Design Lever	Reference
Biomass as a dosage form (oral)	Washing + stabilization/drying	Potency loss due to moisture/temperature and batch compositional variation if conditioning is not controlled	Potency and stability; inter-batch consistency	Control humidity/temperature/stabilization time; define potency and stability criteria on biomass	[25]
Natural metabolites (cyanobacteria)	Extraction + multistage fractionation	Mixture of congeners with closely similar properties; high separation burden and need for analytical selectivity	Identity/purity; release robustness	Design separation alongside the analytical (profiling) strategy to control congeners; avoid feeds with variable profiles	[18]
Pigments/lipophilic metabolites (diatoms)	Pigment recovery and purification	Strong dependence on feed profile and operational stability; culture variation changes separability	Recoverable yield; consistency	Control feed continuity and stability to sustain separation assumptions; select a train compatible with moderate variation	[39]

4. Quality-Oriented Modeling for Scale-Up and Bioprocess Control Strategy

In pharmaceutical biomanufacturing with microalgae and cyanobacteria, modeling and the Quality by Design (QbD) approach become meaningful when they enable transforming photophysical and biological variability into risk-justified design and control decisions because light gradients, CO₂/O₂ constraints, hydrodynamic heterogeneity, and optical fouling do not only shift productivity, but can also shift the regime of product stability and the composition of the matrix entering downstream, such that the central problem ceases to be finding a single good operating point and instead becomes defining a design space and a control strategy that sustain critical attributes under disturbances and scale changes. This logic is supported by the pharmaceutical development framework that links process understanding to design space and knowledge-based control [42]. It becomes more demanding in photosynthetic platforms because operation is governed by physical fields that change with geometry and optical density, requiring that useful models incorporate transport and optical phenomena to achieve comparability of physiological state and product consistency under intensification [43]. In secreted biologics, moreover, because some product loss in secreted systems may happen before capture, coordination among production, harvest, and early recovery should be viewed as both a product-quality and a yield consideration [23,44], whereas in intracellular routes, deterioration of downstream performance may be dominated by transitions of the extract toward emulsions and colloids that collapse clarification and capture, which means that QbD must treat the quality of the feed to separation as a critical output of cultivation rather than as an operational detail [17].

The operational framework of Quality by Design (QbD) is delineated as a sequence that commences with the product's quality target profile, which is then converted into critical attributes and critical process variables that can be quantified or inferred, ensuring that robustness relies on risk-based control and lifecycle performance monitoring rather than final inspection. This way of thinking aligns with quality risk management, which requires identifying hazards, assessing their criticality, and defining controls proportional to risk [2], and with the pharmaceutical quality system, which is oriented to process performance monitoring, knowledge management, and change control [45]. In photosynthetic platforms, this chain becomes operationally distinct depending on the pharmaceutical mode of the product, because the risk hierarchy and the nature of critical attributes do not coincide across a secreted biologic, an intracellular biologic, and a biomass-as-product format in which potency is preserved within the matrix; evidence from oral biomass-based formulations in

Arthrospira shows that the control objective shifts from maximum chromatographic purity toward potency/stability consistency under conditioning, which reorders which attributes and which controls dominate [25]. When the product is a natural metabolite with a mixture of congeners, quality requires identity/purity and chemical-profile consistency. Therefore, QbD must explicitly integrate an analytical strategy to distinguish congeners and assess their sensitivity to process perturbations, because the risk is not only low titer but also profile drift toward species that are difficult to separate or justify [18].

To ensure the validity of the modeling, it must be systematically categorized by decision, differentiating between design models, scale-up models, and control models. In photosynthetic systems, mechanistic growth and productivity models often capture average trends but inadequately predict regime transitions associated with light–shade cycling, oxygen accumulation, or optical fouling—phenomena that specifically induce quality deviations and losses in recoverability. At the design level, the most useful approach is often hybrid: a mechanistic core representing the non-negotiable aspects of the physical system (light attenuation, CO₂/O₂ balance, pH–carbonate coupling, and gas–liquid transfer) combined with empirical layers that capture specific host–product or matrix–separation relationships that are difficult to parameterize and whose form depends on the pharmaceutical product, avoiding naïve extrapolations from flasks or small columns to intensified operation [43]. At the control level, by contrast, models must be identifiable online and observable with available instrumentation; consequently, soft sensors and data-driven models that estimate biomass, pigments, or operating states from spectral signals, process variables, and photophysiological measurements become relevant. Recent reviews explicitly discuss the use of machine learning for prediction and monitoring in microalgal cultures, emphasizing its potential for control and early detection of deviations [46]. Here a critical point emerges that is often omitted and should be made explicit: in photosynthetic systems, the light field and optical fouling alter the signal–state relationship, such that the dominant risk in soft sensing is not only initial error, but drift driven by changes in optics, cell density, and geometry, which compels designing recalibration strategies, drift monitoring, and validation under representative perturbations if these models are to be used as part of a control strategy rather than only as analytical tools.

This requirement translates into a methodological need to deepen the section without duplicating upstream/downstream: QbD requires that the design space incorporate uncertainty and transferability, because a design space that is not robust to scale changes or fouling becomes a laboratory artifact. Risk management provides the language to formalize this, enabling prioritization of variables by criticality and assignment of control levels based on severity and detectability [2]. Still, its technical implementation demands sensitivity analysis and uncertainty propagation to identify which factors dominate the probability of quality deviation, and which can be controlled loosely without increasing risk.

Experimental design, under this approach, must meet two objectives simultaneously: building the design space and generating informative data for model identification and sensor validation, avoiding convenient Designs of Experiments (DoE) that maximize statistical significance but do not improve controllability. QbD logic guides the structuring of DoE based on attributes and risks, because the aim is to link process variation to quality variation, rather than only to productivity, consistent with knowledge-based pharmaceutical development [47]. In photobioreactors, moreover, many factors are not scale-invariant and are mediated by geometry, optical path, and mixing; therefore, DoE intended to remain valid upon scale-up must include descriptors that represent the physical regime and not only nominal setpoints, avoiding design spaces that collapse under intensification.

The connection between DoE/modeling and operation is made through Process Analytical Technology (PAT) within an overall control strategy, aligning with QbD principles

and the regulatory view that product quality should be integrated into the process via timely measurements and process understanding rather than relying solely on end-product testing [48]. In photosynthetic platforms, classical instrumentation becomes insufficient if it does not incorporate optical and photophysiological signals, because distinct metabolic states can coexist with similar biomass under different light fields; in that context, pulse-amplitude modulation (PAM) fluorometry and derived metrics have been proposed as monitoring tools to quantify photosynthetic activity and anticipate performance changes, suggesting their potential as PAT elements when the objective is to sustain consistency rather than to maximize a single-point titer [49]. PAT should not be understood simply as the addition of sensors, but as the use of measurements that are explicitly linked to process decisions, critical attributes, and risk reduction within the control strategy [48]. In photosynthetic platforms, this principle may involve monitoring physicochemical variables alongside optical and photophysiological signals to detect changes in culture state relevant to process consistency and subsequent recovery performance [50–53]. When tools are discussed in downstream, evidence of multi-attribute monitoring using Raman spectroscopy and chemometrics in virus-like particle (VLP) recovery shows that PAT can extend to purification stages and need not be limited to cultivation, providing a conceptual basis for incorporating recovery variables into the control strategy of the full bioprocess. However, in algal matrices, the additional challenge is handling interferences from pigments and amphiphiles [54].

The control strategy that emerges as most robust in photosynthetic pharmaceutical biomanufacturing tends to be hierarchical, combining fast loops on physical and operational variables with slow loops based on state inference and risk-based decision rules, because the objective is not only to hold setpoints but to sustain trajectories compatible with quality. In advanced control, model predictive control approaches are relevant because they manage multivariable systems and constraints, and methodologies for nonlinear predictive control applied to variables such as pH in cultivation systems have been proposed, illustrating that reactive control can be replaced by anticipatory control when a model and measurement are available [55]. In the pharmaceutical context, in secretory production routes, the practical value of process control lies in defining harvest and capture windows that limit supernatant residence time, thereby reducing the risk of extracellular product deterioration before recovery [23,44] and when it enables the avoidance of transitions into operating regimes that amplify colloids/emulsions and fouling, which subsequently deteriorate clarification and capture performance, which connects to the matrix complexity described for algal processing [17]. This control architecture is better sustained when it is integrated with a lifecycle framework that formalizes change management and protocols for post-approval modifications, because accumulated operational evidence feeds refinement of the design space and control strategy over time, consistent with the pharmaceutical quality system and with lifecycle tools for managing changes supported by process understanding [45,56].

The QbD chain is understood as a decision circuit that starts from the target quality profile and translates it into critical quality attributes (CQA) and critical process parameters (CPP), uses DoE to estimate relevant effects and nonlinearities, and combines mechanistic and hybrid models to separate what depends on the physical regime from what depends on the host and the product. This formalization explicitly incorporates uncertainty and scale transferability because optics, mass transfer, and fouling alter the relationship between nominal setpoints and the system's effective state, thereby redefining the risk of quality deviation. The operational closure of this circuit is provided by PAT when available signals allow inference of critical states and activation of control rules, with additional requirements for meteorological validation, drift control, and data governance to ensure soft sensors are usable as part of the control strategy rather than only as diagnostics (Figure 2).

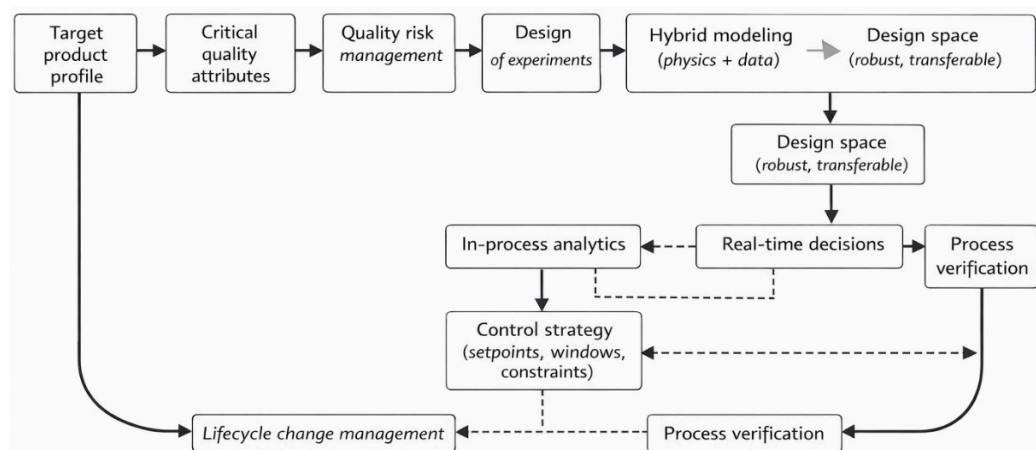


Figure 2. QbD decision flow for design space, PAT, and control strategy.

5. Regulation, TEA/LCA, Challenges and Future Perspectives

In pharmaceutical biomanufacturing based on microalgae and cyanobacteria, the regulatory framework does not operate as a documentary filter that is cleared at the end, but as a design constraint that requires translating biological performance into a verifiable quality system, with traceability and variability control from Chemistry, Manufacturing, and Controls (CMC) development onward. This translation is grounded in quality risk management, because it requires identifying which sources of variation can shift critical attributes and how they are mitigated with risk-proportionate controls, avoiding reactive responses once a batch has already lost quality [2]. At the same time, the product lifecycle requires that scale-up and inevitable process changes be governed through established conditions and change-management tools that preserve comparability, which has a direct implication in photobioreactors, because seemingly operational modifications such as geometry, lighting strategy, mixing regime, or harvest windows can alter the microenvironment and, through that pathway, redistribute physiological states that are reflected in biologic stability or in the chemical profiles of the metabolite [56]. In parallel, the pharmaceutical quality system requires governance, responsibilities, deviation management, and corrective and preventive actions (CAPA), and this architecture is particularly determinative when the process combines biological uncertainty with high downstream sensitivity, because robustness ceases to be an aspiration and becomes a criterion of regulatory acceptability [47]. The practical result is that designing the bioprocess implies defining precisely what is considered controlled, what is considered a change, and what evidence is required to sustain that the process produces comparable material over time, with the same profile of attributes and impurities relevant to safety and efficacy.

This regulatory pressure materializes in Good Manufacturing Practice (GMP) as an operational requirement for facilities, equipment, documentation, and control, such that compliance is not exhausted by testing the final product, but requires the entire process to be reproducible and auditable, including cleaning, prevention of cross-contamination, calibration and qualification, and document governance [45]. This obligation has a specific meaning in photosynthetic platforms because photobioreactors are systems in which performance depends on surfaces, gas–liquid transfer, and thermal control, and these elements interact with biofouling and cleaning routines, which affect both operational stability and traceability of process conditions [57]. In the United States, the CGMP framework requires that manufacturing control be incorporated into the overall production system through documented, verifiable procedures for facilities, equipment, operations, and records, rather than relying solely on end-product testing; for biologics, these standards are supplemented by the specific regulatory requirements in 21 CFR Parts

600–680 (<https://www.ecfr.gov/current/title-21/chapter-I/subchapter-F?utm>, accessed on 22 February 2026).

In biologics, moreover, the expected linkage between process and quality is operationalized by requiring demonstration of control over critical attributes and over the propensity to degradation/variants, which forces integrating monitoring, sampling, and capture/polishing decisions as part of the design, rather than as a later correction when fragments or aggregates appear; this constraint becomes especially visible in secreted routes where the dominant loss can occur before capture due to proteolysis or product instability in the medium. In antibody-producing diatoms, evidence of degradation and variants that condition polishing reinforce the idea that pharmaceutical viability depends on sustaining integrity and a quality profile that is repeatedly reproducible, because downstream processes can amplify quality losses originating upstream if windows and conditions compatible with stability are not designed [34].

In this context, techno-economic assessment (TEA) and life cycle assessment (LCA) add value when they are used as decision instruments rather than as annexes, because they require formalizing which process configurations remain viable when, in addition to yield, the costs and burdens associated with control, cleaning, consumables, and release-related rejects are incorporated. In TEA, the decisive point for photosynthetic platforms is that many economic penalties arise from energy- and consumable-intensive operations—harvesting, concentration, drying, clarification, and polishing—and, although much of the evidence comes from non-pharmaceutical contexts, its conclusions on sensitivity to recovery and stabilization technologies are transferable as an engineering logic when a process architecture consistent with control and quality is sought [20]. For pharmaceutical manufacturing, this sensitivity must be explicitly expanded toward factors that are not usually incorporated in microalgae TEA, but that dominate real decisions, such as nonproductive time due to cleaning and qualification, consumables usage in clarification and membranes/resins, and the cost of nonconformance linked to deviations from the quality profile; this expansion is consistent with the quality-system logic and with change governance across the CMC lifecycle, because cost is not explained only by energy but by controllability and operational repeatability [45,56]. In LCA, the central contribution is to anchor the discussion to functional units consistent with pharmaceutical routes and to system boundaries that do not obscure dominant burdens, because an inventory based on kg of biomass can be inadequate when the true target is the released dose or kg of active ingredient at specification. That choice changes the interpretation of hotspots if the route is intracellular with multiple polishing steps, secreted with membrane-based concentration, or biomass formulation with moisture and microbiological control. Recent LCA evidence for microalgae points consistently to electricity and chemical inputs as relevant hotspots in certain frameworks; therefore, operation selection must incorporate, from conceptual design, strategies to reduce the energy demand of separations and the chemical burdens associated with cleaning and conditioning, especially if the process uses artificial lighting or downstream trains that are intensive in these terms [19].

The technical implication is that the viability of these platforms is largely determined by the coherence among product route, impurity vector, and control strategy, because each configuration shifts the dominant risk and, with it, the relative weight of cost and impact. In secreted routes, reducing suspended solids may ease some early recovery steps, but downstream control still relies on maintaining extracellular product stability and managing dilute product streams during concentration and purification [28,41]. In intracellular routes, matrix complexity and co-extraction of pigments, lipids, and polysaccharides can dominate clarification and membrane/resin performance, such that the separation architecture must be designed to decouple impurities early and minimize fouling and losses driven

by viscosity/emulsions, as is widely documented in reviews of algal processing [17]. In biomass-formulation routes, chromatography reduction reconfigures the problem toward potency, stability, moisture, and compositional consistency under microbiological-control constraints, which makes specification and assay design compatible with a nonconventional format central, because the quality argument is sustained by traceability and evidence of consistency, not by absence of impurities achieved through classical purification [25]. In cyanobacterial metabolites, the bottleneck is often in selectivity and identity/purity relative to congeners; therefore, the separation strategy must be co-designed with the analytical strategy, because demonstrating pharmaceutical consistency depends on chemical traceability and profile control, not only on total titer [18].

From this perspective, operational challenges can be expressed as evidence and instrumentation barriers that currently limit the transition from proof of concept to regulated manufacturing. A structural barrier is demonstrating comparability under changes in scale or lighting configuration, because in photobioreactors heterogeneity in irradiance and dissolved gases can alter the microenvironment and redistribute phenotypes, and this redistribution manifests downstream as variability in stability or chemical profiles; therefore, modeling and monitoring must serve to anticipate stable operating regions and to detect drift before it translates into quality loss [58]. Another barrier is the lack of broadly adoptable minimal analytical packages for photosynthetic matrices, because without robust identity/potency/impurity panels that operate consistently in algal extracts or biomass, batch release becomes fragile and industrial transfer slows, even if upstream production is stable.

As future perspectives, the first axis is consolidating monitoring and sensing that enable inferring culture state and quality trends in near-real time, with explicit management of model drift and continuous validation, because in photosynthetic systems the relationship among optical signals, physiology, and quality is not static and changes with scale and operating regime; recent evidence in sensing and monitoring reinforces the need for this discipline for robust control [50]. Within this framework, the verifiable criterion is to demonstrate a robust correlation between monitored variables and critical attributes of the product or extract that anticipate downstream losses, and that this correlation is maintained after planned lifecycle [56]. A second axis is strengthening modeling–control integration through hybrid approaches (first-principles models with data-driven components), not to predict maximum yield, but to bound operating regions that minimize physiological drift and sustain consistency, as evaluated by demonstrating performance stability under perturbations rather than only under nominal conditions [58]. A third axis is producing TEA/LCA specifically formulated for GMP scenarios with transparent assumptions that incorporate cleaning cycles, consumables, nonproductive times, reject rates, and verification, because these elements can reorganize hotspots and change architecture decisions and are currently underrepresented in the open literature.

On the other hand, the pharmaceutical maturity of microalgae and cyanobacteria is defined by the ability to sustain, under reproducible operation, a causal and traceable linkage between process conditions, impurity profile, and critical product attributes, such that variability inherent to photosynthetic systems is translated into a scientifically justified operating space and a risk-proportionate control strategy, with change governance across the CMC lifecycle [2]. Within this framework, viability does not depend on maximizing single-point titers, but on coherently integrating upstream–downstream architecture, analytical capability, and change control to preserve product comparability under scale-up and planned modifications in lighting, mixing, harvesting, or capture, avoiding reliance on final testing alone to demonstrate quality [56]. Operational consistency must be evaluated together with its economic and environmental penalty, because cleaning cycles, consum-

ables, nonproductive times, and release requirements can reorganize the dominant factors in TEA and hotspots in LCA; therefore, route selection and its control strategy must remain compatible with a complete process inventory and with system boundaries consistent with a compliant product rather than with biomass.

6. Conclusions

Pharmaceutical production using microalgae and cyanobacteria should be viewed as an integrated bioprocess challenge where product type and location determine the recovery method, impurity levels, and analytical efforts needed to obtain compliant material. In this framework, upstream operation cannot be defined solely by nominal cultivation setpoints because effective light exposure, CO₂/O₂ management, mixing, and optical fouling influence physiological states that directly impact active fractions, product stability, and the composition of the material entering separation. Downstream performance is then dictated by the primary loss mechanism associated with each route. Intracellular products require coordinated disruption and clarification to limit emulsions, colloids, and membrane or resin fouling. Secreted products demand recovery strategies aligned with product stability in the supernatant to reduce extracellular degradation and variant buildup. Biomass-based formulations shift control toward potency, moisture, storage stability, and microbiological consistency. Products rich in structural analogs need separation and analytical methods designed together to maintain identity and purity with traceability. This review's main contribution is to present microalgae and cyanobacteria as pharmaceutical process platforms instead of only photosynthetic hosts evaluated by expression capacity. This view is important because it connects platform choice, upstream conditions, recovery design, and quality strategy within a unified engineering approach. Consequently, route evaluation focuses not just on productivity but on whether the process remains controllable, comparable, and manufacturable during scale-up and changes. This is especially relevant because biological feasibility has advanced faster than the process understanding needed to ensure pharmaceutical quality in heterogeneous phototrophic operations. Three main gaps still hinder regulatory robustness and industrial adoption. First, models rarely include biological quality attributes explicitly under gradients and perturbations, limiting the ability to quantitatively understand how photophysical changes lead to potency loss, aggregation, degradation, or variants. Second, regulatory manufacturing requirements are not fully represented in techno-economic and environmental assessments, especially regarding cleaning, validation, consumables, nonproductive time, and release rejects, even though these factors influence cost structure and process hotspots when aiming for compliant material rather than biomass. Third, the field lacks minimal, reproducible, and interlaboratory analytical panels tailored to product class and matrix, which are crucial for comparability, batch release, and lifecycle management. Addressing these three issues is essential to turn promising photosynthetic systems into reliable pharmaceutical manufacturing routes that are technically manageable and meet quality standards.

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