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Review

Phycobiostimulants 2.0: Integrating Algal Biomass Provenance, Processing, Molecular Mechanisms and Predictable Crop Performance

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Abstract

Phycobiostimulants derived from macroalgae, microalgae, and cyanobacteria are increasingly used to improve crop performance. However, their effects vary across products, crops, and environments. In this review, we propose a biomass–process–composition–mechanism–efficacy (BPCME) framework to systematically trace this variability. Biomass provenance, including taxonomic identity, cultivation conditions, physiological state, and harvest timing, defines the initial biochemical potential, whereas processing and formulation determine the molecular profile delivered to the plant. The current findings indicate that phycobiostimulants act mainly by modulating endogenous signaling networks, hormone crosstalk, redox regulation, metabolic reprogramming, and rhizosphere and plant–microbiome responses rather than by direct nutrient or hormone replacement. Agronomic performance is further moderated by the genotype, dose, application route, developmental stage, soil conditions, and environmental stress. To improve reproducibility, we propose a phycobiostimulant quality-by-design (QbD) framework that integrates candidate critical biomass attributes and process parameters, chemical fingerprints, biological potency, and batch equivalency. Cascading algal biorefineries and precision application strategies can improve resource efficiency while helping to define the conditions for reliable field performance. Progress towards precision phycobiostimulants will require the experimental linking of product identity and mechanism to reproducible potency across batches and agronomic environments.

Keywords: phycobiostimulants; algal biomass; biostimulant standardization; chemical fingerprinting; quality-by-design; precision agriculture

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1. Introduction

Recent research on phycobiostimulants has emphasized product characterization, mechanisms of action, and function-specific evaluation [1,2]. Earlier studies have largely established biological activity without resolving its chemical basis [3], whereas recent studies have integrated chemical profiling with physiological, molecular, and metabolomic analyses to relate product composition to plant responses [4,5]. Reproducibility across products and agronomic conditions remains a major practical challenge [6]. A recent meta-analysis of 198 studies and 2021 treatment–control comparisons reported a mean 16.3% increase in yield or biomass (95% CI: 13.5–19.1%). However, the magnitude of the response varies with the application strategy, formulation, algal source, extraction method, crop group, and edaphic conditions [6].

A major source of variability is product identity [1,7]. Terms such as "seaweed extract" or "microalgal biostimulant" indicate the origin but do not define the applied product; preparations from the same source may differ in composition, processing history, and formulation [1,8]. Two *Ascophyllum nodosum* biostimulants differed approximately fourfold in polyphenol content and altered 4.47% and 0.87% of the measured *Arabidopsis* transcriptome, respectively [7]. Microalgal species cultivated under standardized conditions produce extracts with distinct biochemical signatures and species-, crop-, and dose-dependent responses [5]. Five commercial seaweed extract formulations, applied at a common concentration, produced distinct germination and seedling responses in *Capsicum annuum* [9], while growth, biomass accumulation, and root traits were also dose-dependent [10].

Product identity reflects the combined effects of biomass provenance and processing. Species or strain, physiological state, cultivation environment, and harvest conditions determine the initial biochemical potential [8,11], whereas processing determines which constituents are retained, modified, or concentrated [12]. For *A. nodosum*, composition varies with harvest location, season, physiological status, and manufacturing procedure, whereas bulk descriptors such as total solids and ash provide limited information on biologically relevant constituents [8]. *A. nodosum* preparations produced under different alkaline-processing conditions showed distinct molecular weight profiles and different capacities to protect tomato reproductive development under heat stress [12].

Biological interpretation requires adequate characterization of the applied material [2,8]. Phenotypic responses demonstrate activity but cannot distinguish whether responses arise from native biomass constituents, processing-derived fractions, interactions among components, or modulation of endogenous plant regulation [8]. The interpretation of microalgal and cyanobacterial products is further complicated by the diverse functional constituents, including phytohormones, polysaccharides, soluble amino acids, and components associated with nitrogen-fixing activity [13]. Coupling chemical characterization with biological response profiling can help distinguish putative bioactive constituents from experimentally validated links between composition, signaling pathways, and phenotypes [4]. Shukla et al. [1] provided a broad synthesis of phycobiostimulants as sustainable agricultural input. The present review extends this synthesis by linking biomass provenance and processing with product composition, mechanistic evidence, biological potency, and agronomic efficacy within the BPCME continuum.

This review uses a biomass–process–composition–mechanism–efficacy (BPCME) continuum to establish traceability from source biomass to agronomic performance [6,7,11,12]. The framework also incorporates response moderators, quality-by-design (QbD), and cascading algal biorefineries. Although the BPCME continuum is integrative, not every link has been experimentally validated. Evidence is stronger for the effects of biomass provenance and processing on product composition, whereas causal links from specific chemical features to molecular mechanisms and from controlled potency to reproducible

field performance are less established. As illustrated in Figure 1, the BPCME continuum begins with algal biodiversity and cultivation conditions that define biomass provenance, followed by extraction, fractionation, and formulation, which determine the chemical fingerprint. It then links product composition with plant perception and rhizosphere interactions, signaling and transcriptomic, proteomic, and metabolomic responses, functional traits such as root architecture, nutrient use, photosynthesis, and stress priming, and field performance. Quality control, standardization, safety, and circular biorefineries operate across this sequence, while Table 1 summarizes the current evidence status and principal validation gaps across the five BPCME components.

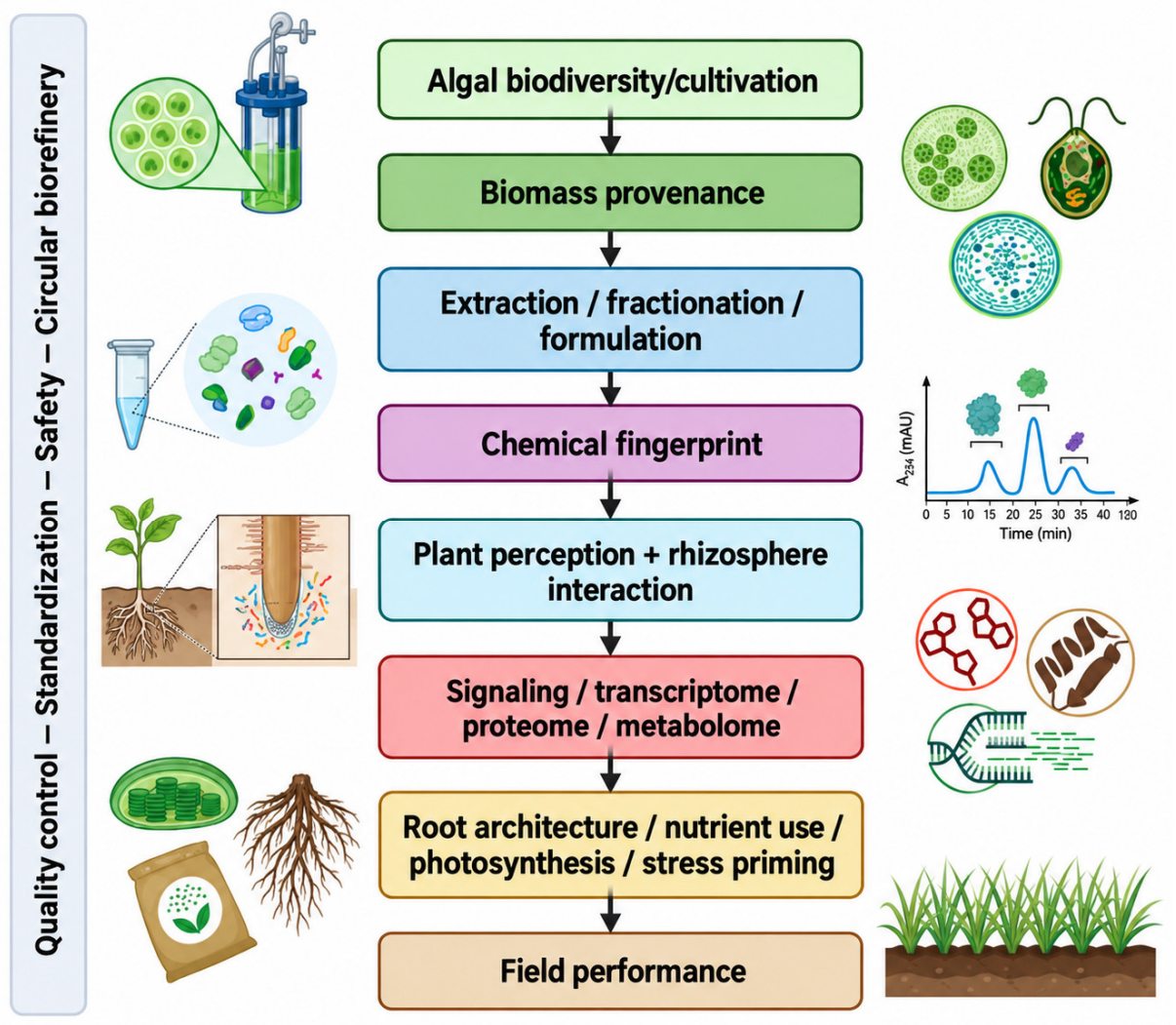


Figure 1. Biomass–process–composition–mechanism–efficacy (BPCME) continuum for phycobiostimulants.

Safety and sustainability are cross-cutting qualification criteria rather than considerations applied only after efficacy has been demonstrated. Biomass provenance determines contaminant and resource-use profiles; processing may remove, transform, or concentrate undesirable constituents while altering energy and material demand; and agricultural qualification requires safety assessment and system-level sustainability evaluation. These considerations are particularly important for wastewater-derived and stranded biomass, where the feedstock origin introduces variability in contaminants, microbiological risks, traceability, and resource recovery benefits.

Table 1. Current evidence status and major validation gaps across the BPCME continuum.

BPCME Component	Current Evidence	Evidence Status	Main Remaining Gap	References
Biomass provenance	Taxon/strain, cultivation conditions, geographic origin, physiological state, and harvest timing alter the biomass composition and biological activity.	Experimentally supported	Direct demonstration that controlled provenance produces a defined fingerprint and reproducible potency across batches and field environments	[11,14–28]
Processing	Extraction conditions, cell disruption, hydrolysis, fermentation, fractionation, and formulation modify the chemical recovery, molecular structure, and biological activity.	Experimentally supported	Quantitative process parameters → chemical attributes → potency relationships and validated operating ranges.	[12,29–40]
Composition	Chemical and metabolomic profiling can discriminate products and identify candidate bioactive fractions or constituents.	Partially resolved	Identification of functionally relevant markers, bioavailability, mixture interactions, and potency-linked acceptance ranges.	[7,39,41–43]
Mechanism	Defined glycans and selected fractions have receptor-proximal, genetic, transcriptomic, metabolomic, or physiological support, whereas complex extracts are mainly supported by association or omics evidence.	Mixed; causal evidence limited	Receptor identification, experimental perturbation, fraction-recombination studies, and causal testing of rhizosphere-mediated effects	[39,44–69]
Efficacy	Controlled trials, multi-environment studies, and meta-analyses confirm agronomic activity but also substantial heterogeneity across products, crops, application strategies, and environments.	Supported but context-dependent	Batch-controlled, multi-location, and multi-year validation across genotype × environment × management combinations, including neutral and negative responses.	[6,9,55,70–77]

Scope and Literature Search Strategy

This review focuses on phycobiostimulants derived from macroalgae, microalgae, and cyanobacteria, covering biomass provenance, processing, chemical composition, biological mechanisms, agronomic efficacy, standardization, safety, and precision application. Relevant literature was identified using Scopus, Web of Science, PubMed, and Google Scholar. The review focused mainly on studies published from 2016 to August 2026, while earlier studies were included selectively when they provided foundational mechanistic or methodological evidence. Search terms included combinations of "phycobiostimulant", "algal biostimulant", "seaweed extract", "microalgae", "cyanobacteria", "plant biostimulant", "extraction", "chemical composition", "mechanism", "crop performance", and "standardization". Primary experimental studies were prioritized, together with relevant reviews, meta-analyses, and regulatory documents. The evidence was synthesized as a structured narrative review according to the BPCME framework.

2. Defining Phycobiostimulants: Source, Product Form and Functional Boundaries

In this review, the term "phycobiostimulant" is used as a source-based scientific descriptor for plant biostimulant products derived from macroalgae, eukaryotic microalgae, cyanobacteria, or their processed products [1]; it does not represent a separate regulatory product class [78,79]. Under the EU functional definition, plant biostimulants stimulate plant nutrition processes independently of nutrient content, thereby improving nutrient-use efficiency, tolerance to abiotic stress, quality traits, and nutrient availability in the soil or rhizosphere [78,79]. Phycobiostimulant preparations may contain nutrients, viable cells, antimicrobial compounds, and biostimulant-active components [1,13]. Therefore, their classification depends on the primary demonstrated or claimed function rather than source composition alone. Throughout this review, "macroalgae" refers to seaweeds, "microalgae" to eukaryotic microalgae, and cyanobacteria are identified separately because of their distinct taxonomy and functional characteristics. "Source biomass" collectively refers to

biomass from all three groups, whereas "algal biomass" refers to macroalgal or eukaryotic microalgal biomass. "Extract" refers to a processed preparation from the source biomass, whereas "phycobiostimulant" refers to the final plant-biostimulant preparation, which may consist of whole biomass, viable cells, extracts, fractions, or formulated products.

Macroalgal aqueous extracts and formulated products can differ substantially from intact biomass in terms of composition and activity [1,80]. Thus, the term "seaweed extract" does not imply chemical or functional equivalence among products [1]. Intact cells, disrupted biomass, intracellular extracts, and extracellular culture products are chemically and biologically distinct. Alling et al. [81] compared intact cells, mechanically disrupted cells, and cell-free culture supernatants of *Chlorella vulgaris* and *Tetradesmus obliquus* (syn. *Scenedesmus obliquus*) and found preparation- and species-dependent effects on the germination of tomato and barley. Likewise, extracts from different microalgal and cyanobacterial species have produced distinct effects on tomato growth, nutrient uptake, and metabolite profiles [82], while suspensions of seven strains have produced significantly different cucumber germination kinetics and seedling vigor [14]. Thus, "microalgal biostimulant" is an incomplete product descriptor unless the taxon, strain, and preparation type are specified in the label.

Cyanobacteria differ from eukaryotic microalgae taxonomically and functionally, as their agricultural applications include non-living extracts, viable preparations, and diazotrophic inocula [82,83]. *Limnospira platensis* (syn. *Arthrospira platensis*) extracts have been evaluated as non-living biostimulants in grapevine [84], whereas nitrogen-fixing cyanobacteria can contribute directly to crop nitrogen and act as biofertilizers [82]. Viable microalgae and cyanobacteria are being explored as biological inputs for hydroponic systems [84]. Under saline hydroponic conditions, viable microbial biofertilizers, including microalgae, influence ion homeostasis, osmotic adjustment, antioxidant responses, and phytohormonal regulation [85]. Thus, the functional category of a cyanobacterial product depends on cell viability and the biological functions retained after processing.

Phycobiostimulants are functionally distinct from nutrient fertilizers, microbial biofertilizers, and plant protection products, although individual formulations may exhibit overlapping effects [78,79]. Nutrient fertilizers act principally by supplying plant nutrients, whereas microbial biofertilizers rely on viable microorganisms that increase nutrient availability and acquisition [86]. Products intended primarily for pest or pathogen control fall within plant protection or biocontrol frameworks; antimicrobial activity alone does not classify a product as biopesticide [78,79]. Suspensions of *Nostoc* sp. BCAC 1226 exhibited antifungal activity against phytopathogenic fungi and oomycetes [87], illustrating that cyanobacterial preparations may exhibit biological activities beyond plant growth stimulation.

We conceptualize this transition as a progression from "Phycobiostimulants 1.0" to "Phycobiostimulants 2.0" (Figure 2). "Phycobiostimulants 1.0" refers to source-defined and largely empirically evaluated products identified mainly by algal origin and product form, with efficacy assessed primarily from phenotypic responses and limited integration of biomass provenance, processing history, chemical composition, biological potency, and mechanistic evidence. In contrast, "Phycobiostimulants 2.0" refers to functionally specified products developed through controlled biomass provenance and processing, chemical fingerprinting, standardized biological potency, mechanism-informed evaluation, and validation within defined genotype × environment × management conditions. These terms are conceptual developmental categories and are not formal regulatory or chronological classifications.

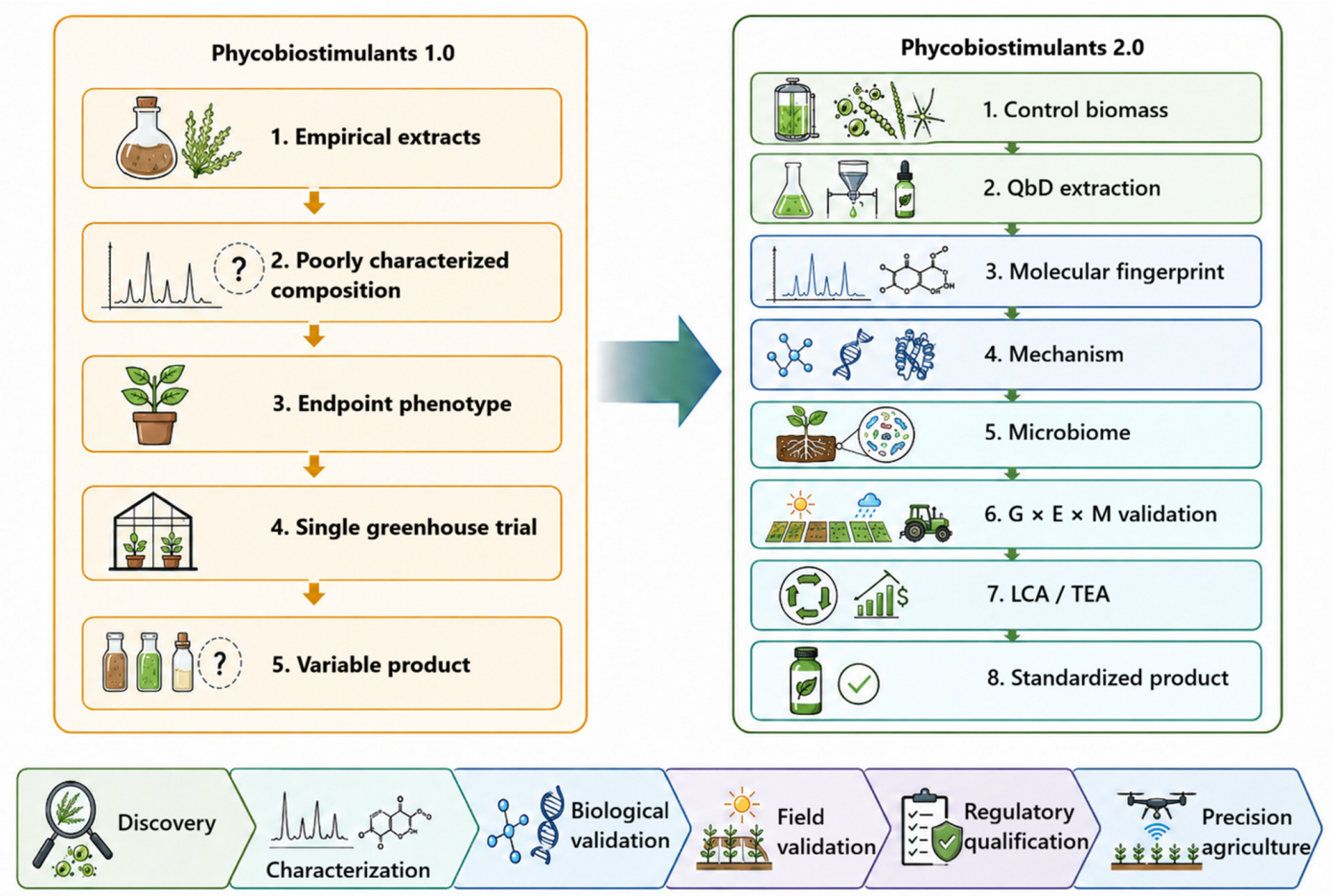


Figure 2. Conceptual transition from empirically defined "Phycobiostimulants 1.0" to functionally specified "Phycobiostimulants 2.0". Abbreviations: QbD, quality-by-design; G × E × M, genotype × environment × management; LCA, life-cycle assessment; TEA, techno-economic assessment.

3. Biomass Provenance as the First Determinant of Phycobiostimulant Identity

3.1. Taxonomic Identity, Strain and Geographic Provenance

Phycobiostimulant identity begins with biomass entering the processing stage. Species name alone is insufficient because genetic background and production environment influence biochemical composition. Significant interspecific differences in carbohydrates, proteins, ash, and minerals occur among seaweeds [15], whereas *Ulva* strains grown under similar conditions can differ in growth, nutrient uptake, and composition [16]. Strain-level differences can also affect biological activity; standardized suspensions of microalgal and cyanobacterial strains produced significantly different germination and seedling vigor responses in cucumber [14]. Geographic origin also contributes to variability; *Saccharina latissima* cultivated across Norwegian coastal sites showed site-, depth-, and season-dependent compositional variation [17]. Thus, taxon, strain or isolate, and production location are distinct provenance factors.

3.2. Cultivation Environment and Physiological State

Controlled cultivation can reduce biomass variability while enabling control of composition. Nutrient supply, irradiance, and temperature modify algal metabolism and biochemical allocation. Nitrogen or phosphorus limitation alters antioxidant activity and carotenoids, phenolics, tocopherols, and ascorbic acid in *Phaeodactylum tricornutum*, *Tetraselmis suecica*, and *C. vulgaris* in a species-dependent manner [18]. Light intensity and spectral composi-

tion affect the accumulation of lipids, carbohydrates, and carotenoids [19–21]. Irradiance alters lipid and carbohydrate accumulation in *C. vulgaris*, whereas carotenoid production in *Jaagichlorella luteoviridis* depends on light conditions and nutrient availability [20,21]. Heat exposure can induce metabolomic reprogramming in *Ulva* holobionts [22]. Therefore, the relevant production target is not merely biosynthetic capacity but reproducible expression of a defined chemical fingerprint under controlled physiological conditions.

The cultivation medium may also influence downstream biological activity. *C. vulgaris* MACC-1 biomass produced in BG-11 medium showed modest biostimulant activity, whereas wastewater-grown biomass showed no detectable activity in the germination assay but greater antimicrobial activity [23]. Thus, cultivation history can alter downstream functions even within the same strain, and biomass produced from different nutrient sources cannot be assumed to be functionally equivalent.

The growth phase adds further variation. Comparative metabolomics of *Chlorella* and *Parachlorella* showed reprogramming between exponential and stationary phases, including changes in amino acids, photosynthesis-related metabolites, and lipid accumulation, although responses were strain-dependent [24]. The irradiance on carbohydrate, lipid, and chlorophyll accumulation also varies with the growth phase [20]. Thus, the physiological state at harvest provides a more meaningful biomass specification than culture duration alone.

3.3. Harvest Timing and Post-Harvest Stabilization

Seasonal provenance is important for macroalgal species distribution. Long-term profiling of kelps has revealed seasonal shifts in laminarin, mannitol, alginate, protein, ash, minerals, and polyphenol content [11]. These shifts can influence downstream agricultural performance, as extracts prepared from monthly harvested *A. nodosum*, *Fucus serratus*, and *Fucus vesiculosus* produced harvest-month-dependent plant responses, with effects varying according to the crop and extract concentration [25]. Thus, harvest timing is a production variable rather than descriptive collection metadata.

Drying methods alter the chemical and physicochemical properties of *Ulva* sp. and *F. vesiculosus* [26]. Changes can begin before stabilization: short-term seawater storage of freshly harvested *Alaria esculenta* and *S. latissima* altered dry matter and compositional attributes within hours [26], whereas longer storage under inappropriate conditions caused loss of oxidation-sensitive constituents [27]. Therefore, the time to stabilization, washing, drying, storage conditions, and final moisture content are parts of the biomass specification before extraction.

3.4. From Biomass Variability to Cultivation-by-Design

These observations support the cultivation-by-design concept, in which an intended agronomic function is linked to a target biochemical profile, appropriate strain, cultivation regime, and defined physiological state at the time of harvest [18,23]. Such an approach could convert algal metabolic plasticity from a source of batch variability into controllable production attributes. Cultivation-by-design requires an experimentally demonstrated chain of culture conditions → biomass fingerprint → biological potency, rather than the optimization of biomass productivity or metabolite concentration alone. However, direct evidence linking deliberately engineered biomass composition to reproducible crop efficacies remains limited. Thus, cultivation-by-design remains a testable framework, rather than an established production strategy. For wild macroalgae, an analogous strategy is provenance-based selection using authenticated source populations, defined collection areas and seasonal harvest windows [11,17].

4. Processing as Biological Engineering: From Algal Biomass to Functionally Defined Products

After harvesting and stabilization, processing determines which constituents and molecular structures are included in the final product. Temperature, pH, solvent environment, mechanical energy, and hydrolysis can alter the solubility, structural integrity, and recovery of phenolics, peptides, lipids, carbohydrates, and other metabolites [12,29,30]. Two *A. nodosum* preparations produced from a common source under different processing conditions showed distinct molecular weight profiles and capacities to protect tomato reproductive development from heat stress [12]. Thus, processing can alter the composition and biological activity and is an integral part of phycobiostimulant identity rather than a neutral recovery step.

4.1. Extraction Severity and Selectivity

Extraction performance is better evaluated using chemical profiles and biological potency than using techniques or yields alone. Aqueous extraction depends on temperature, pH, residence time, and solid-to-liquid ratio. In *F. vesiculosus*, extraction pH and enzyme-assisted processing altered recovery of sugars, phenolics, and several plant growth regulators. Relative to conventional water extraction, enzyme-assisted extraction increased neutral sugars by 34%, total phenolics by approximately twofold, and selected cytokinins by several-fold [30].

Polysaccharides are sensitive to extraction severity, as processing can modify their molecular size, conformation, sulfation, and structural integrity. In *Ulva* spp., ultrasound extraction under increasingly acidic conditions shifted ulvan from predominantly >800 kDa material to lower-molecular-weight fractions, consistent with partial acid-induced depolymerization [31]. Microwave extraction of carrageenan from *Sarcopeltis skottsbergii* showed a trade-off between solubilization and structural preservation: greater severity increased recovery but promoted molecular degradation, whereas milder conditions retained higher molecular weight, sulfate-containing structures [32]. Extraction depends on process parameters such as time, temperature, pH, enzyme dose, and energy input. The optimal regime depends on whether the desired product requires intact polymers, defined oligomers, or a broader soluble fraction [30–32].

Cell disruption determines access to intracellular constituents in microalgae and cyanobacteria. Mechanical and physical treatments differ in terms of rupture efficiency, chemical recovery, and biological activity. Stirk et al. [29] showed that disruption methods alter extract yield, antioxidant properties, and plant biostimulant activity. Freeze-drying, sonication, and ball milling increased recovery to different extents; however, a greater yield did not consistently correspond to greater plant activity. Thus, mass recovery, potency per unit extract, and, where available, activity per unit starting biomass are separate measures of process performance.

Pulsed electric fields can release intracellular materials. In *Chlorella*, PEF increased the recovery of proteins, polyphenols, and other intracellular constituents, whereas comparisons with high-pressure homogenization indicated preferential release of soluble or low-molecular-weight fractions [33,34].

Supercritical CO_2 extraction uses CO_2 above its critical temperature and pressure, combining gas-like diffusivity with liquid-like solvating capacity. In a typical system, compressed CO_2 is pumped through an extraction vessel containing biomass, where the target compounds dissolve in the supercritical phase. Depressurization in downstream separators reduces solvent power and releases the extracted compounds. Pressure, temperature, CO_2 flow rate, extraction time, biomass particle size, and co-solvent addition are major variables controlling extraction efficiency and selectivity. Because supercritical CO_2

is relatively nonpolar, modifiers such as ethanol can improve the recovery of more polar constituents. Advantages include tunable selectivity, relatively low extraction temperatures, limited organic-solvent residues, and simple solvent removal. Limitations include high-pressure equipment, capital and operating costs, and relatively poor recovery of highly polar compounds unless a modifier is used. In *Gigartina pistillata*, pressure, temperature, and ethanol concentration influence the recovery of lipids, phenolics, carotenoids, and polyunsaturated fatty acids [35]. At 15 MPa, 50 °C, and 10% ethanol, conditions favored total yield and phenolic recovery, whereas higher pressure favored carotenoid recovery [35]. Therefore, its value for phycobiostimulant production depends on biological potency and process economics.

4.2. Hydrolysis, Fermentation and Fractionation

Hydrolysis converts proteins or polysaccharides into smaller peptides, amino acids, or oligosaccharides, making the extent and pattern of depolymerization more informative than the label "hydrolyzed." Controlled *Ulva* processing illustrates this distinction: ultrafiltration generates ulvan-enriched fractions, whereas subsequent microwave/H_2O_2 depolymerization shifts the material toward lower molecular weights [36]. However, reduced molecular size alone does not demonstrate increased biostimulant potency without biological validation.

Fermentation can transform product composition because microbial metabolism and enzymes modify phenolic, amino acid, and volatile profiles [37]. Solid-state fermentation of *Kappaphycus* alters these chemical classes [37]. Fermentation of freshly harvested *Oedogonium intermedium* generated extracts with different nutrient compositions and plant growth-promoting activity in mung beans, with the most effective preparation producing greater dry biomass than comparator products [38]. Thus, fermentation-derived compositional changes form part of the final product identity.

Fractionation is useful for linking chemistry and bioactivity. Membrane-based separation, solvent or salt precipitation/partitioning, and chromatographic approaches can separate extract components according to molecular size, charge, polarity, and solubility [36,39,88]. These approaches improve causal attribution but potentially disrupt mixture-dependent effects. Sequential fractionation of an *A. nodosum* extract produced a lipophilic fraction enriched in fatty acids and sterols that retained freezing-protective activity and was associated with specific transcriptomic and metabolomic responses in *Arabidopsis* [39]. Bioactivity-guided fractionation can connect chemical fingerprints with biological activity, although activity in an enriched fraction does not establish a single causal compound.

4.3. Stabilization and Formulation

The composition can continue to change during concentration, drying, formulation, and storage. Spray drying is scalable but introduces thermal exposure, whereas encapsulation can protect labile constituents and alter their delivery. In *Saccharina japonica*, spray-drying wall material influenced phenolic encapsulation efficiency, with whey protein and gelatin achieving approximately 87% [40]. Therefore, carriers and encapsulants can contribute to formulation identity and require controls to separate their effects from those of the active preparation. Concentration, drying conditions, storage parameters, and stability are additional formulation characteristics.

Extraction yield alone is insufficient for optimizing the process. Stirk et al. [29] showed that greater recovery after microalgal cell disruption did not correspond to greater plant biostimulant activity. A multi-objective framework considers the recovery, chemical fingerprint, structural integrity, biological potency, stability, energy demand, and cost. The principal biomass–process–composition relationships and analytical requirements are listed in Table 2.

Table 2. Biomass provenance–processing–composition relationships governing phycobiostimulant identity.

Algal Species	Product Form	Provenance/Process Variable	Main Compositional Effect	Process	Functional Implication	Recommended Characterization	Reference
Brown kelps: *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima*, *Alaria esculenta*	Whole biomass	Species and season	Seasonal shifts in laminarin, mannitol, alginate, protein, ash, minerals and polyphenols	Harvest/stabilization	Harvest window changes the chemical starting material	Moisture, ash, C/N, minerals, major carbohydrates, polyphenols	[11]
S. latissima	Whole biomass	Site, depth and season	Protein, tissue N, nitrate, ash and yield	Cultivation/harvest	The same species can yield chemically different biomass across production sites	Location/date, culture conditions, protein/N, nitrate, ash, elemental profile	[17]
Ulva spp.	Whole biomass	Strain and season	Growth, starch, fiber, protein, lipid and ash	Cultivation/harvest	Strain selection alters feedstock quality even under similar conditions	Strain authentication, proximate composition, growth phase, harvest date	[16]
Phaeodactylum tricornutum, *Tetraselmis suecica*, *Chlorella vulgaris*	Whole biomass	N/P availability	Species-specific changes in carotenoids, phenolics, ascorbate and tocopherols	Controlled cultivation	Supports cultivation-by-design; crop efficacy not tested	Nutrient status, growth phase, pigments, antioxidants, metabolite fingerprint	[18]
C. vulgaris MACC-1	Whole biomass/derived preparation	Inorganic medium vs. wastewater	Altered physiological state and downstream bioactivity	Cultivation + extraction	Cultivation substrate can change biostimulant activity	Medium chemistry/contaminants, dry matter, fingerprint, potency bioassay	[23]
Ulva sp.; *Fucus vesiculosus*	Whole biomass	Drying method	Changes in nutrients, pigments, antioxidants and physicochemical properties	Convective, microwave-vacuum or freeze-drying	Post-harvest stabilization forms part of product identity	Moisture, drying history, pigments, antioxidants, proximate composition	[26]
Ascophyllum nodosum	Characterized extract	Processing severity	Different molecular-weight/compositional profiles	Low- vs. high-temperature alkaline processing	Direct process → bioactivity evidence: different heat-stress protection in tomato	pH–temperature–time history, dry matter, MW distribution, carbohydrate profile, potency assay	[12]
Chlorella spp.; *Scenedesmus acutus*	Derived extracts	Cell-disruption method	Differences in yield, antioxidants and plant-active components	Freeze-drying, sonication, ball milling	Direct process → bioactivity evidence: increased extract recovery did not predict plant-biostimulant activity	Disruption energy/time, yield, fingerprint, antioxidant and plant bioassays	[29]
Ulva spp.; *Sarcopeltis skottsbergii*	Extracted/defined polysaccharide material	pH, ultrasound amplitude and microwave temperature	Polymer recovery, molecular-weight distribution and structural/rheological changes	Ultrasound or microwave extraction	Process controls polysaccharide structure; crop potency not established	Energy/time/pH, MW distribution, FTIR/NMR, sulfate, monosaccharides	[31,32]
Commercial *A. nodosum* extracts	Commercial products	Same nominal source, different manufacturing	~Fourfold difference in polyphenols plus broader compositional differences	Commercial processing	Chemical identity → response evidence: 4.47% vs. 0.87% of the *Arabidopsis* transcriptome altered	Dry matter, polyphenols, chemical fingerprint, transcriptomic or potency assay	[7]

Abbreviations: MW, molecular weight; FTIR, Fourier-transform infrared spectroscopy; NMR, nuclear magnetic resonance; C/N, carbon-to-nitrogen ratio; N/P, nitrogen-to-phosphorus ratio.

5. From Chemical Fingerprints to Plant Molecular Reprogramming

The chemical fingerprint of a phycobiostimulant guides its biological analysis. However, the composition alone does not establish a mechanism. Seaweed extracts contain complex mixtures of polysaccharides, phenolics, amino compounds, compatible solutes, lipids, pigments, minerals, and hormone-like molecules, the abundance and biological relevance of which vary among products [41,89]. Metabolomic profiling can identify candidate compounds; however, annotation alone does not establish their contributions to plant responses. Biological interpretation is strengthened by linking a defined chemical feature or fraction with early perception or signaling, downstream regulation, and phenotypes [89].

5.1. Chemical Complexity and the Identity of the Active Signal

Algal polysaccharides and oligosaccharides provide evidence of direct signaling mechanisms [44–47]. Laminarin/β-1,3-glucans, ulvan, and alginate oligosaccharides demonstrate that chain length, polymer structure, and chemical substitution can influence biological recognition processes. Wanke et al. [47] showed that long- and short-chain β-1,3-glucans are perceived differently among plant species, and that recognition of long-chain glucans depends on the degree of polymerization. Ulvan and alginate oligosaccharides have been linked to jasmonate- and auxin-associated responses, respectively [45,46]. Thus, biological activity can depend on molecular architecture rather than abundance of a carbohydrate class.

The specific contributions of other constituents, including phenolics and betaines, to ANE signaling and plant responses remain unclear [89]. The metabolomic characterization of *Kappaphycus alvarezii* extracts identified candidate bioactives associated with reported biological functions [41]. However, these associations do not establish which constituents are responsible for plant responses [41,42].

5.2. Perception, Redox Signaling and Early Regulatory Events

Algal glycans provide the strongest receptor-proximal evidence. Laminarin induces Ca^{2+} influx, extracellular alkalinization, oxidative burst, MAPK activation, and defense gene expression in grapevines [44]. β-Glucan recognition differs among plant species and between long- and short-chain glucans [47]. Wanke et al. [47] showed that long-chain β-glucan recognition in some species is independent of CERK1, supporting the existence of multiple recognition systems rather than a single conserved receptor. However, these findings cannot be directly extrapolated to chemically complex commercial products. Laminarin and ulvan have primarily been investigated as defense elicitors, and their mechanisms cannot be assumed to explain the growth promotion by complex extracts [44,45].

Redox responses require interpretation in relation to timing, localization, and physiological context. *A. nodosum*-based SuperFifty altered ROS-associated genes, programmed cell death/autophagy pathways, and metabolite and lipid profiles, and protected *Arabidopsis* from paraquat injury [48]. Pretreatment attenuated paraquat-induced transcriptional responses and modified the expression of growth-, hormone-, carbohydrate-, and antioxidant-related genes. These responses support redox-associated priming but do not identify the initiating molecules or their receptors.

Transcriptomic enrichment of MAPK or hormone-signaling pathways indicates pathway association, not direct activation of a specific kinase or receptor. A commercial *A. nodosum* extract alters genes associated with MAPK signaling, hormone signal transduction, glutathione metabolism, and other pathways in tomato. However, receptor binding and MAPK phosphorylation were not measured [49]. Direct pathway activation or necessity requires biochemical or genetic perturbation.

5.3. Phytohormone Crosstalk Rather than Hormonal Replacement

The view that seaweed extracts act mainly by supplying auxins or cytokinins is insufficient to explain many product responses. Wally et al. [50] quantified phytohormones in a commercial *A. nodosum* extract and found that amounts delivered at the recommended application rates were insufficient to explain the observed responses directly; treatment instead modified endogenous phytohormone biosynthesis and accumulation [50,89].

Pathway perturbation studies provide stronger mechanistic evidence. Olaetxea et al. [51] showed that growth promotion by a complete *A. nodosum* extract was impaired in *Arabidopsis* lines altered in salicylic acid, auxin, and cytokinin signaling. Several individual constituents and a reconstructed mixture were biologically active, but no single constituent reproduced the whole-extract response. This supports interactions among extract components and coordinated salicylic acid, auxin, and cytokinin signaling [51] rather than a single-hormone mechanism.

Microalgal extracts induce species-dependent transcriptional responses in lettuce, involving auxin- and cytokinin-associated pathways, transcription factors, amino acid and carbohydrate metabolism, and cell cycle regulation [90]. Because the pathway necessity and initiating compounds were not tested, these responses remain omics-supported associations rather than causal mechanisms.

5.4. Metabolic Reprogramming and the Emergence of Phenotype

Nair et al. [39] combined transcriptomic and metabolomic analyses with mutant analysis and showed that freezing protection by a lipophilic fraction of *A. nodosum* required stress-associated proline and soluble sugar accumulation; protection was lost in *p5cs1* and *sfr4* mutant backgrounds defective in these responses. This provides stronger causal evidence than pathway enrichment alone.

Algal treatments also alter carbon, nitrogen, sulfur, and ion homeostasis. Jannin et al. [91] combined microarray and physiological analyses to identify changes in N, C, and S metabolism in *Brassica napus*. Dell'Aversana et al. [92] showed that *A. nodosum*-based treatment under salinity altered amino acid metabolism and K^+ levels, consistent with ion homeostasis, osmotic adjustment, and ROS scavenging in tomato. Time-resolved metabolomics revealed tissue- and time-dependent changes in amino acids, lipids, phytohormones, organic acids, phenylpropanoids, and other metabolites following seaweed-extract treatment of *Arabidopsis* [52]. However, metabolite abundance alone is insufficient to infer pathway flux; Schwender et al. [53] found that less than half of the reactions showing significant differences in flux showed a significant change in the corresponding substrate or product. Therefore, claims of altered metabolic throughput require isotope tracing or metabolic flux analysis.

Drought pretreatment with Super Fifty induced physiological and transcriptomic responses consistent with stress priming, including maintenance of tissue water status and shoot-apical-meristem activity during subsequent drought [54]. These findings support physiological and transcriptional priming but not persistent or transgenerational stress memory, which requires evidence beyond the immediate treatment–stress sequence.

A study on pepper and eggplant integrated yield assessment, transcriptomic analysis, metabolomic analysis, and elemental analysis associated with *A. nodosum* treatment revealed changes in carbohydrate and nitrogen metabolism, stress responses, and increased fruit yields [55]. This provides field-level evidence linking molecular responses to productivity. However, the initiating molecules and cross-location reproducibility remain unclear.

5.5. A Biological Evidence Hierarchy for Phycobiostimulants

The evidence level is assigned to a specific mechanistic claim rather than to the study as a whole. Association refers to co-occurrence or correlation between treatment, molecular changes, and phenotype without experimental intervention. A candidate mechanism requires a defined compound, fraction, or product feature linked to a temporal or dose-related signaling, metabolic, or physiological response but does not demonstrate pathway necessity. An omics-supported mechanism requires coordinated transcriptomic, proteomic, metabolomic, or multi-omics changes that support a defined pathway and are linked to the phenotype, without direct perturbation of that pathway. Causal evidence requires experimental intervention in the proposed mechanism through pathway inhibitors, mutants, genetic perturbation, depletion or addition of a defined constituent, or loss- and rescue-of-response experiments, with a corresponding phenotypic change.

The hierarchy reflects evidential strength rather than technological complexity; a multi-omics study may remain associative, whereas a targeted mutant or loss-of-response experiment can demonstrate pathway necessity. Current evidence for chemically complex commercial extracts is concentrated at the candidate- and omics-supported levels. Receptor-proximal evidence is strongest for defined glycans, whereas functional genetic evidence remains limited [39,47,51]. Antioxidant enzyme activity, differential gene expression, pathway enrichment, and metabolite accumulation do not constitute proof of mechanism.

A. nodosum evidence supports modulation of endogenous regulatory networks rather than direct phytohormone supply [50,89]. The representative evidence and major uncertainties are summarized in Table 3.

Table 3. Mechanism–evidence matrix distinguishing purified compounds, defined fractions, complex extracts, and commercial formulations, and linking these material types with plant perception, signaling, molecular reprogramming, physiological outcomes, and strength of biological evidence.

Bioactive/Fraction	Material	Proposed Action/Early Signaling	Molecular/Functional Evidence	Endpoint/Model	Evidence Strength	Major Uncertainty	Reference
Laminarin/β-1,3-glucans	Purified/defined polysaccharide	Early elicitor signaling involving Ca^{2+} influx, alkalinization, ROS and MAPKs; recognition depends on glucan chain length and plant species	Defense-gene induction; comparative recognition studies show species-dependent β-glucan perception	Defense activation and reduced pathogen infection; grapevine and multiple model species	Strong defined-elicitor/perception evidence	Cognate receptors remain unresolved for several β-glucan responses, distinct recognition systems, and CERK1-independent perception of long β-glucans in some host species.	[44,47]
Ulvan	Purified/defined polysaccharide	Sulfated-polysaccharide elicitation linked mainly with JA signaling	Defense-expression profile and *Arabidopsis* hormone-mutant evidence	Defense activation; *Medicago* and *Arabidopsis*	Pathway-supported/mutant evidence	Receptor and structure–activity relationships within heterogeneous ulvan remain unresolved	[45]
Alginate oligosaccharides	Defined oligosaccharides	Ca^{2+}-associated stimulation of endogenous auxin biosynthesis/transport	Increased *OsYUCCA*, *OsIAA* and *OsPIN1* expression and IAA; response inhibited by TIBA and EGTA	Root development; rice	Strong pathway-perturbation evidence	Receptor identity and relevance to crude alginate-containing products remain unknown	[46]
Whole *Ascophyllum nodosum* extract	Complex whole extract/commercial preparation	Modulation of endogenous hormone networks rather than sufficient direct hormone supply	Product hormone levels insufficient to explain activity; response reduced in SA-, auxin- and cytokinin-impaired lines; individual components failed to reproduce whole-product activity	Growth regulation; *Arabidopsis*	Strong pathway evidence for a complex extract	Initiating molecules and component interactions remain unresolved; crop validation is limited	[50,51]
C. vulgaris and *Scenedesmus quadricauda* extracts	Non-purified extracts	Species-dependent auxin- and cytokinin-associated regulation	Transcriptomic changes in hormone pathways, transcription factors, carbohydrate/amino-acid metabolism and cell cycle	Growth-related responses; lettuce	Omics-supported candidate mechanism	Active compounds and pathway necessity were not established	[90]
Lipophilic *A. nodosum* fraction	Defined/enriched fraction	Metabolic priming of freezing-response pathways	Transcriptomics + metabolomics; freezing protection lost in mutants impaired in stress-associated proline/sugar accumulation	Freezing tolerance; *Arabidopsis*	Omics + functional genetic evidence	Initiating bioactive molecule and receptor remain unknown	[39]
A. nodosum-derived SuperFifty	Commercial characterized extract	Redox/stress priming rather than simple ROS scavenging	Transcriptomic, metabolomic and lipidomic changes involving ROS, PCD/autophagy, hormones and primary metabolism	Paraquat-stress protection; *Arabidopsis*	Multi-omics-supported mechanism	Initiating signal and necessity of individual pathways remain unresolved	[48]
Kappaphycus alvarezii-based formulation	Commercial characterized product	Foliar treatment associated with plant-mediated restructuring of the rhizosphere	16S rRNA amplicon profiling, Tax4Fun/FAPROTAX functional prediction and soil-enzyme responses	Growth and altered rhizosphere composition; maize	Systems-level microbiome association	Causal route through root exudation or other plant-mediated processes remains unproven	[56]
Commercial *A. nodosum* extract	Commercial product	Coordinated molecular reprogramming under field conditions	Metabolomics + transcriptomics + elemental analysis linked treatment with N assimilation, carbohydrate metabolism and osmoprotection	Increased yield; pepper and eggplant, two-year field study	Translational systems-level evidence	Initiating molecule/receptor and multi-location reproducibility remain unresolved	[55]

Note: Evidence categories: association, co-occurrence without intervention; candidate mechanism, defined constituent or fraction linked to a mechanistically relevant response; omics-supported mechanism, coordinated molecular evidence supporting a pathway without direct perturbation; causal evidence, experimental perturbation demonstrating necessity and/or sufficiency of the proposed mechanism. Abbreviations: ROS, reactive oxygen species; MAPK, mitogen-activated protein kinase; JA, jasmonic acid; SA, salicylic acid; IAA, indole-3-acetic acid; TIBA, 2,3,5-triiodobenzoic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; PCD, programmed cell death; 16S rRNA, 16S ribosomal RNA; FAPROTAX, Functional Annotation of Prokaryotic Taxa.

6. Rhizosphere as the Second Functional Target of Phycobiostimulants

Phycobiostimulant action extends beyond the plant to the rhizosphere, where effects may arise through plant-mediated and direct routes [56–58]. Plant-mediated effects arise when treatments modify plant metabolism or root chemistry, thereby altering microbial recruitment or activity. Direct effects occur when soil-applied constituents or viable algal/cyanobacterial cells interact with the microorganisms or soil matrix. These routes are distinct but can occur simultaneously. Hines et al. [57] demonstrated direct stimulation of arbuscular mycorrhizal fungi, together with enhanced plant accommodation of symbiosis, whereas Selvaraj et al. [58] identified both direct bacterial responses to *A. nodosum* extract and stronger root exudate-mediated effects. Distinguishing these routes requires route-specific controls, including plant-free product exposure, foliar application without soil contact, and isolated root exudate or microbial assays.

6.1. Plant-Mediated Recruitment and Rhizosphere Assembly

Root exudates link plant physiological state to rhizosphere assembly. In *Avena barbata*, developmental changes in root exudate chemistry interact with bacterial substrate preferences to generate reproducible patterns of microbial community assembly [59]. Foliar application of a *K. alvarezii*-derived biostimulant altered the bacterial composition of the maize rhizosphere [56]. Because α-diversity did not consistently increase despite taxonomic redistribution, the response is better described as community restructuring rather than a general increase in microbial diversity.

Selvaraj et al. [58] showed that root exudates from *A. nodosum* extract-treated maize promoted *Pseudomonas protegens* CHA0 motility and altered the genes involved in chemotaxis (*cheW, cheV*), pyoverdine synthesis (*pvdS*), pyrrolnitrin production (*prnD*), and HCN synthesis (*hcnA*). Treatment also altered the rhizosphere bacterial composition and increased maize root benzoxazinoids, including DIMBOA and MBOA. Product-only controls altered some *P. protegens* genes, but motility responses were stronger to exudates from treated plants, supporting concurrent direct product–microbe and indirect plant-mediated signaling [58]. The specific exuded metabolites responsible remain unresolved.

6.2. Direct Microbial Responses and Soil Physical Effects

Soil application enables direct microbial exposure, although planted soil experiments cannot distinguish direct effects from root-mediated responses. A fermented *Sargassum horneri* fertilizer altered bacterial α-diversity, community composition, and several soil enzyme activities in tomato field soil [60]. However, plants prevent specific attribution to the direct stimulation of microbes. An alkaline *A. nodosum* extract increased *Rhizophagus irregularis* spore germination, germ-tube length, and hyphal branching in vitro, and soil drench and foliar treatments enhanced mycorrhizal establishment in *Medicago truncatula* [57].

A. nodosum extract and fucoidan increased several plant growth-promoting activities of *P. protegens* CHA0, including siderophore production, phosphate solubilization, and expression of selected chemotaxis and secondary metabolite genes [61]. Combinations of bacteria with plant extracts or their components have also been shown to improve pea growth under saline conditions [61]. These findings establish product–microbe functional interactions but do not demonstrate that microbial response is necessary for plant phenotypes.

Viable microalgae and cyanobacteria can modify soil physical properties through filamentous growth and production of extracellular polymeric substances (EPS). In agricultural soil microcosms, indigenous photosynthetic microbial communities increase macroaggregation and mean-weight diameter, associated with bound EPS and filamentous enmeshment of soil particles [62]. Chamizo et al. [63] showed that *Phormidium ambiguum* or *Scytonema javanicum* inoculation produced biocrusts, modified EPS or soil C/N depending on strain,

and improved aggregate stability across soil textures. These mechanisms depend on viable phototrophs and, therefore, do not necessarily apply to nonliving algal extracts.

6.3. From Community Restructuring to Ecological Function

Community shifts are agronomically meaningful only when they alter the functions relevant to plant performance. Seaweed biostimulant treatments have been associated with changes in microbial abundance, soil enzyme activity, nutrient availability, and crop performance [56,60,64]. Microbial and enzyme changes have been associated with increased tomato yield [60], whereas altered bacterial communities have coincided with increased available soil N after seaweed extract application [64]. However, these associations do not establish a causal direction or quantify the microbially mediated nutrient fluxes.

Functional profiles inferred from 16S-marker data represent predicted genomic potential rather than direct measurements of microbial transcription, protein activity, or biogeochemical flux [65]. Nivetha et al. [56] used Tax4Fun and FAPROTAX to infer N-, P-, S-, and chemotaxis-related functions. Such predictions represent functional proxies rather than direct evidence of microbiome-mediated crop improvement.

Hellequin et al. [66] found that a seaweed- and amino-acid-based soil biostimulant altered active bacterial and fungal communities in bare soil and increased active saprophytes and putative plant-growth-promoting bacteria after 49 days, but did not increase soil organic-C mineralization. Thus, community activation or compositional change does not necessarily translate to predicted ecological function.

Renaut et al. [67] reported that a commercial *A. nodosum* extract altered bacterial and fungal communities associated with tomato and pepper and identified taxa correlated with increased productivity. Because community membership was not experimentally manipulated, these taxa remain candidate contributors rather than causal determinants of productivity.

6.4. Rhizosphere Conditioning and the Evidence Required for Causality

The term "rhizosphere priming" has been used for phycobiostimulant-induced microbiome responses [56], but "rhizosphere conditioning" is more appropriate when only short-term post-treatment changes are observed. Persistent priming or ecological memory requires altered community structure or function to persist after treatment withdrawal and influence subsequent challenges or recolonization. Therefore, Nivetha et al. [56] support rhizosphere restructuring or conditioning but not persistent ecological memory.

Amplicon sequencing demonstrated community restructuring, whereas predicted metagenomes and soil enzyme measurements provided functional information. Chemotaxis, microbial gene expression, and plant-free assays provide direct evidence of microbial responses. Background agronomic management can modify soil cyanobacterial and microalgal community structures. DNA metabarcoding has shown changes in community diversity under different fertilizer intensities [68]. Causal involvement of phycobiostimulant-induced microbiome changes requires experimental manipulation linking microbial composition or function to plant phenotype [69]. Gnotobiotic systems, synthetic communities, depletion–rescue, microbiome transplantation, and selective exclusion can be used to test microbial necessity and sufficiency [69].

Current evidence supports community restructuring, direct microbial responses, stimulation of arbuscular mycorrhizal development, and viable phototroph-mediated changes in soil structure. However, microbiome-mediated causality for plant phenotypes remains limited and requires experimental manipulation to show that altered microbial communities or functions change the corresponding plant response.

7. From Biological Activity to Predictable Field Performance: Understanding Response Moderators

Agronomic efficacy is not an intrinsic property of phycobiostimulants. Product identity can be represented conceptually as PI = f(B × Pr × F), where biomass provenance (B), processing (Pr), and formulation (F) define the applied product. Crop response can then be represented as R = f(PI × G × Env × M), where genotype (G), environment (Env), and management (M) moderate efficacy; these expressions denote interacting determinants, rather than predictive equations. A meta-analysis of 198 studies and 2021 treatment–control comparisons reported a mean 16.3% increase in yield or biomass (95% CI: 13.5–19.1%), but responses varied with experimental setting, formulation, application strategy, crop, and environment [6]. Controlled-environment studies averaged 21.8% compared with 14.0% in field trials, and soil-directed applications showed higher subgroup means than foliar treatments [6]. These subgroup differences identify potential response moderators but do not establish the superiority of a particular application strategy. The pooled mean does not represent a uniform expected response for an individual product or production environment. Subgroup estimates are based largely on comparisons among different studies rather than randomized comparisons of moderators within the same experiment; differences among subgroups may reflect confounding factors as well as true biological moderation. Thus, the pooled effect indicates overall activity, while prediction of product-specific performance requires effect estimates within more narrowly defined product × genotype × environment × management domains.

Formulation-dependent variability is evident under standardized conditions. Five commercial seaweed extract formulations applied at the same concentration to *C. annuum* produced distinct germination, root growth, and biomass responses, although independent chemical characterization and field validation were not included [9]. The translational objective was to define a domain of reproducible performance rather than a universally optimal treatment.

7.1. Dose, Timing and Delivery Define the Exposure Window

The dose cannot be interpreted independently of the application route, crop stage, and stress context. In water-stressed tomatoes, *A. nodosum* extract produced dose- and route-dependent effects, with 5 mL L^{-1} providing the most favorable response under both soil drench and foliar application, although the magnitude of the response differed between routes [70]. Thus, the nominal concentration is meaningful only within the specific route × environmental context.

The phenological stage and timing relative to stress also modify efficacy. Trivedi et al. [71] compared ten *K. alvarezii* application schedules across vegetative and grain-filling stages in maize and showed that stage selection influenced response. In soybean, application of *A. nodosum* three days before drought or at the onset of 50% water-holding capacity produced different physiological and biochemical responses [72]. Pretreatment had a stronger effect on nitrate reductase activity, chlorophyll retention, and lipid peroxidation, whereas treatment at drought onset influenced other antioxidant and stomatal responses. The concentration, frequency, developmental stage, and timing relative to stress define the exposure window. The meta-analysis found no significant linear relationship between applied concentration and yield, although early-stage programs involving four to six applications included some of the more consistent positive responses [6].

The delivery route further determines the initial site and physiological context of exposure. In grapevines under progressive water deficit, foliar *A. nodosum* treatment improved gas exchange and water-use efficiency under mild-to-moderate stress and supported faster photochemical recovery after rewatering, whereas soil application produced limited responses

under the same conditions [73]. Such within-experiment comparisons provide stronger evidence of route dependence than cross-study contrasts. Pérez-Oñate et al. [6] reported higher pooled responses for fertigation (20.6%) and drenching (19.0%) than for foliar application (15.5%). However, these subgroups do not establish a universal ranking, and route-dependent performance remains specific to the product–crop–environment combination.

7.2. Genotype and Stress Context Modify Responsiveness

Genotype remains an under-examined response moderator. The same seaweed extracts increased runner yield by 19% in strawberry 'Fortuna' but by 8% in 'Albion', while feeder-root density increased significantly only in 'Fortuna' [74]. Field evidence from eggplant showed similar cultivar dependency: among cultivars treated with an *A. nodosum*-based product, significant improvements in early yield and fruit number occurred primarily in 'Flavine' F1 [75].

A. nodosum treatment alleviated the effects of water deficit in both 'Arbequina' and 'Galega' olives, but through cultivar-specific physiological and molecular responses [76]. 'Arbequina' showed stronger phenolic and antioxidant responses, whereas 'Galega' exhibited distinct changes in antioxidant enzymes, metabolites, lipids, and gene expression. These findings support genotype × treatment interactions rather than extrapolation from a single cultivar to an entire crop species.

Environmental stress is a quantitative response moderator. Labels such as "drought," "salinity," or "heat" conceal variations in intensity, duration, onset, developmental stage, and recovery conditions. Ahmed et al. [70] evaluated 50%, 75%, and 100% field capacities; Cordeiro et al. [72] distinguished treatments before and at the onset of water restriction; and Dias et al. [76] maintained olive trees at 50% field capacity for 69 days. Stress severity, duration, onset, and recovery provide more informative response descriptors than binary stressed/non-stressed categories.

7.3. From Controlled Efficacy to a Domain of Reliable Performance

In this review, "predictable performance" does not imply a universally positive response. It refers to the ability to prospectively estimate magnitude and uncertainty of a treatment effect within a defined product × genotype × environment × management domain. Quantitatively, predictability can be evaluated using predefined criteria, such as an agronomic effect size, confidence, prediction intervals, treatment × environment variance, and the probability that the response exceeds a specified agronomic threshold. Thresholds are product- and use-specific and therefore belong in the target product profile rather than as universal values.

Controlled experiments are valuable for resolving individual treatment variables but provide limited evidence of performance robustness because environmental and management variations are constrained. Arioli et al. [77] analyzed seven field experiments across five locations, three Australian states, and four wine-grape cultivars. The recurring soil application of a seaweed extract derived from *Durvillaea potatorum* and *A. nodosum* increased the pooled yield by 14.7%. Only one individual experiment showed a statistically significant treatment effect, although all trials showed a positive response; the pooled mixed-model analysis identified an overall effect. This separates single-trial significance from cross-environment reliability as different measures of efficacy.

A reliable performance domain is defined by product identity, cultivar, dose, timing, delivery route, soil and nutrient status, stress intensity, and meteorological conditions. Multi-location and multi-year evidence is most informative when effect sizes and uncertainties for individual environments are considered alongside pooled responses and, where supported by the design, treatment × environment interactions. Arioli et al. [77] reported

cultivar-dependent economic benefits, indicating that biological reproducibility and economic reliability are related but distinct translational endpoints. Positive, neutral, and inconsistent responses define the boundaries of reliable performance.

8. Phycobiostimulant Quality-by-Design: From Variable Biomass to Reproducible Function

Phycobiostimulant identity is often defined mainly by biological source, although products from the same algal taxon can differ chemically and biologically [7,12,42]. European standardization of plant biostimulants has advanced through CEN/TC 455 "Plant Biostimulants", established to support Regulation (EU) 2019/1009. Its program covers sampling, validation of product claims, pathogenic and non-pathogenic microorganisms, safety parameters, and labeling and denominations [93]. Thirty-three European standards have been developed, including the EN 17700 series [94] for substantiation of plant-biostimulant claims. Specifically, EN 17700-1:2024 [94] specifies the general principles, requirements, and quality criteria for trials used to justify such claims. These standards provide a basis for harmonized evaluation, whereas the QbD framework proposed here provides a complementary development approach linking biomass provenance and processing with chemical fingerprints, biological potency, and batch equivalence. ICH Q8(R2) defines QbD as a systematic approach based on predefined objectives, product and process understanding, and process control. Its principal elements are the Quality Target Product Profile (QTPP), critical quality attributes (CQAs), material attributes, critical process parameters (CPPs), risk assessment, design space, and control strategy [95–98]. Here, these principles are adapted without implying pharmaceutical or regulatory equivalence.

8.1. Define the Target Product Profile

Development begins with an agronomically adapted target product profile defined by intended function and use context. A product intended for root establishment under nutrient limitation may require different specifications than one intended for drought priming or reproductive-stage heat tolerance. The target profile specifies the intended biological response, crop or crop group, application route, dose range, stress or nutritional context, storage form, and minimum performance criterion [95–98].

This approach extends the needs-driven biostimulant development framework of Povero et al. [99], which integrates agronomic needs with raw material selection, chemical and biological characterization, product development, and field testing. For phycobiostimulants, this can be expressed as intended function → target product profile → biomass specification → process control → chemical specification → biological potency → performance qualification. Therefore, product specifications are function-driven rather than selected solely for analytical convenience.

8.2. Link Biomass and Process Attributes to Product Quality

We use critical biomass attributes (CBAs) for starting material properties that measurably influence product quality or potency, including authenticated taxon or strain, physiological state, harvest window, moisture, and contaminant status. CBA is a phycobiostimulant-specific extension of the QbD concept of material attributes and is not a formal ICH category [95–98]. A biomass attribute is critical only when its variation is experimentally demonstrated or identified through risk assessment to affect product quality or potency. A process parameter is designated as critical only when variation within a relevant range measurably affects a predefined quality or potency attribute [95–98].

CQAs characterize algal formulations more precisely than nominal algal content or total solids. Depending on product type, candidate CQAs may include molecular weight dis-

tribution, polysaccharide characteristics, phenolic or peptide profiles, marker metabolites, mineral composition, pH, electrical conductivity, and physical stability. Sharma et al. [42] showed that particle size, zeta potential, FTIR, thermogravimetric analysis, and Py-GC/MS differentiated six commercial biostimulant formulations, and that dry matter, density, and chemical composition varied across production batches. Gandhi et al. [43] identified distinct HR-MS and GC-MS metabolite profiles among *K. alvarezii*, *Gracilaria debilis*, and *Sargassum cinctum* extracts. These studies demonstrated analytical discrimination; however, a chemical fingerprint becomes a meaningful CQA only when experimentally linked with biological potency or another predefined product objective.

An operating space linking CBAs and CPPs with CQAs and potency defines the combinations of biomass attributes and process parameters that preserve chemical specifications and biological activity. Until these relationships are experimentally validated, numerical limits remain provisional rather than established critical ranges. A practical QbD could begin with a defined *A. nodosum* biomass lot in which harvest season, collection location, moisture, and physiological state are recorded as candidate CBAs. The extraction pH, temperature, and residence time can then be varied as candidate CPPs. The resulting preparations would be characterized for CQAs, such as molecular-weight distribution, carbohydrate profile, phenolic content, and selected chemical fingerprints, and tested using the same standardized potency assay. Preparations meeting predefined chemical and biological acceptance ranges would be evaluated across selected cultivars, locations, and environmental conditions. This design would test CBA → CPP → CQA → biological potency → agronomic performance. Existing studies support individual links in this sequence, including processing-dependent changes in molecular weight profiles and heat-stress protection [12], batch-dependent chemical variation [42], rapid biological assays for *A. nodosum* preparations [100], and multi-environment field evaluation [77]. However, the complete sequence has not been demonstrated within a single phycobiostimulant development program.

8.3. Add Biological Potency to Chemical Specification

Chemical similarity does not guarantee functional equivalence. Conversely, minor analytical differences do not necessarily indicate functional non-equivalence when biological potency remains within predefined acceptance limits. For complex extracts, biological potency can be defined as the magnitude of a predefined biological response relative to a qualified reference batch or reference preparation under standardized assay conditions. A potency assay includes a reference preparation, negative control, defined dose range, predefined biological endpoint, adequate replication, and acceptance limits. Where a dose–response relationship can be established, potency may be expressed as relative potency or a response-based parameter, such as an ECx or benchmark dose. Assay validation should establish intra- and inter-assay precision and distinguish reference material from deliberately altered or sub-potent preparations. For complex products where no single endpoint adequately represents intended function, a small panel of complementary bioassays may be appropriate, provided each endpoint is linked to the target product profile.

Rayorath et al. [100] evaluated two *A. nodosum* extracts using *Arabidopsis* root-tip elongation, liquid growth, and greenhouse assays and showed that *Arabidopsis* can provide a rapid system for assessing bioactivity of seaweed extracts and fractions. However, this study did not establish a formally validated batch-release method.

High-throughput phenotyping and omics can support identification of sensitive biological endpoints during product development [101], but are not necessarily suitable as routine-release assays. For routine quality control, a fit-for-purpose potency assay should be reproducible, dose-responsive, and capable of distinguishing an acceptable reference prod-

uct from altered or sub-potent products. Its purpose is to detect biologically meaningful variation among batches.

8.4. Batch Equivalence as the Operational Endpoint

The practical endpoint is batch equivalence rather than exact molecular identity. Here, "batch equivalence" refers to chemical, safety, and functional consistency within predefined acceptance limits, not regulatory equivalence in the pharmaceutical sense. Shefer et al. [102] provided a cross-production chemical comparison. Crude ulvan extracted from *Ulva* biomass in 2018 and 2020 using the same process showed similar FTIR and biochemical characteristics, although several elemental concentrations differed. Because only the 2018 extract was evaluated in plant experiments, this study demonstrates chemical similarity across production periods but not biological equivalence between batches.

Few phycobiostimulant studies have compared independent production batches using both chemical fingerprints and potency assays. Sharma et al. [42] compared the chemical characteristics across three production batches, whereas Rayorath et al. [100] demonstrated rapid biological assays for different *A. nodosum* preparations. Integrating both approaches within the same batch-comparison design would provide a direct test of the functional equivalence.

We propose four criteria for batch equivalence: (i) conformity with predefined biomass and process specifications, (ii) a chemical fingerprint within an established acceptance range, (iii) compliance with contaminant and physical stability limits, and (iv) biological potency within a validated response window. Under this control strategy, deviations trigger investigation of biomass provenance, processing history, or formulation, rather than relying on end-product testing alone. Field validation then determines whether controlled product attributes translate into reproducible agronomic performance. Therefore, batch consistency and field reliability represent distinct manufacturing and agronomic validation endpoints. Table 4 summarizes the proposed minimum reporting and QbD requirements.

Table 4. Proposed minimum reporting requirements and quality-by-design framework for phycobiostimulant research and product development.

Reporting/QbD Element	Minimum Reporting/Characterization	Why It Matters	Framework Role	Reference
Algal identification and provenance	Accepted name; strain/isolate; identification method; wild/cultivated status; production or collection site; batch; medium/water source	Taxon alone does not capture strain- or location-dependent variation	Candidate CBA	[16,17]
Cultivation conditions	Medium/nutrient source; N/P status; salinity; pH; temperature; irradiance/photoperiod; CO_2; reactor/pond; culture density	Cultivation environment can alter metabolite allocation and downstream activity	Candidate CBA/cultivation input	[18,23]
Harvest stage/date	Growth phase for cultured biomass; season/date and tissue stage for macroalgae	Harvest timing can alter carbohydrates, proteins, minerals and bioactives	Candidate CBA	[11,16]
Post-harvest handling/storage	Washing; time to stabilization; drying conditions; storage temperature/duration; final moisture	Product chemistry may change before extraction	Candidate CBA/CPP	[26]
Extraction/processing	Biomass: solvent ratio; solvent; pH; temperature; time; enzyme dose; mechanical/ultrasound/microwave energy; fractionation; concentration/drying	Processing controls recovery, degradation, molecular size and potency	Candidate CPP	[12,29,31]
Product strength	Dry matter/total solids; density for liquids; moisture for powders	Enables meaningful dose normalization and product comparison	Product specification/candidate CQA	[6]
Chemical fingerprint	Bulk composition plus function-relevant fingerprint/markers; LC-MS, GC-MS, NMR, SEC/HPSEC, FTIR or targeted analyses as appropriate	Species identity or total solids alone cannot demonstrate chemical equivalence	Product specification/candidate CQA	[7]
Contaminant analysis	Heavy metals and source-relevant biological/organic contaminants, especially for waste-derived biomass	Circular feedstocks may introduce safety risks	Safety specification/candidate CQA	[15,103]
Formulation and stability	Carrier/excipients; preservative; pH/EC; physical form; encapsulation; storage; shelf life; marker and potency stability	Formulation can alter stability, delivery and efficacy	Formulation/process attribute + stability CQA	[104]
Dose	Product concentration and absolute amount delivered; preferably dry-matter- or marker-normalized; multiple doses where appropriate	Dose contributes to response heterogeneity and may be non-linear	Response moderator	[6]
Application method/timing	Foliar, drench, fertigation or seed treatment; spray volume; number/interval of applications; developmental stage	Route and timing define exposure and influence response magnitude	Response moderator	[6]
Crop genotype/cultivar	Species; cultivar/genotype; plant source; developmental stage	Genotype-dependent perception and physiology may alter efficacy	Response moderator	[74,76]
Soil/substrate properties	Texture/type; pH; EC/salinity; organic matter; available nutrients; baseline fertility; fertilizer regime	Edaphic conditions can modify product availability and crop response	Response moderator	[6]
Stress definition/intensity	Stress type, onset, duration, severity, recovery period and quantitative environmental values	Binary "stressed/non-stressed" descriptions are not reproducible	Response moderator	[2,12]
Experimental design/replication	Experimental unit; biological *n*; independent experiments; randomization/blocking; controls; carrier control; statistical model	Controls pseudoreplication and separates algal from formulation effects	Evidence-quality requirement	[6]

Table 4. *Cont.*

Reporting/QbD Element	Minimum Reporting/Characterization	Why It Matters	Framework Role	Reference
Physiological/biochemical endpoints	Function-relevant measures such as gas exchange, fluorescence, water status, ion balance, nutrient uptake and root traits	Confirms response but should not automatically be presented as a mechanism	Biological potency attribute	[12,55]
Molecular validation	Relevant genes/proteins/metabolites; omics where justified; inhibitor, reporter or mutant evidence for causal claims	Distinguishes association from pathway involvement or necessity	Biological validation	[39,51]
Agronomic yield/quality	Marketable yield, yield components, relevant quality traits, effect size and uncertainty	Vegetative responses do not necessarily translate into commercial benefit	Performance endpoint	[6,55]
Multi-year/location/batch evidence	Year/season, site and product batch; report individual and pooled responses where possible	Tests robustness across environment, management and batch variability	Translational qualification	[42,77,102]
Biological potency/batch-equivalence assay	Function-linked rapid assay; reference batch; dose–response; assay precision; predefined acceptance interval	Chemical similarity alone cannot establish functional equivalence	Biological potency attribute	[100,101]

Abbreviations: CBA, critical biomass attribute; CPP, critical process parameter; CQA, critical quality attribute; LC-MS, liquid chromatography–mass spectrometry; GC-MS, gas chromatography–mass spectrometry; NMR, nuclear magnetic resonance; SEC, size-exclusion chromatography; HPSEC, high-performance size-exclusion chromatography; FTIR, Fourier-transform infrared spectroscopy; EC, electrical conductivity.

9. Phycobiostimulants Within Circular Algal Biorefineries

Algal origin alone does not establish sustainability because cultivation, harvesting, dewatering, stabilization, extraction, transportation, and residue management can contribute to environmental burdens [105–107]. A circular framework is a cascading algal biorefinery, in which phycobiostimulant production is integrated with the sequential recovery of higher-value fractions, followed by energy or nutrient recovery. The objective is not to maximize the number of co-products but to preserve the quality and functionality at each stage. Primary studies combining food ingredients, biostimulants, and bioenergy have demonstrated feasibility [108–110].

9.1. Cascading Valorization of Algal Biomass

Villaró et al. [108] recovered a protein isolate containing approximately 91% protein from *L. platensis* for food applications and evaluated the residual fraction as a biostimulant. The residue stimulated adventitious root formation and showed cotyledon expansion and chlorophyll retention, demonstrating that recovery of a high-value protein did not eliminate the agricultural functionality of the residual biomass.

Stepwise processing of *Chlorella sorokiniana* through maceration, high-pressure homogenization, and enzymatic hydrolysis generated fractions that were screened and recombined at optimized concentrations. The resulting blend increased *Arabidopsis* root elongation by 25% and supported tomato growth with a 25% reduction in mineral fertilizer input, while approximately 45% of starting biomass remained available for further valorization [109]. Although further use of this biomass has been proposed rather than demonstrated, this study supports partial rather than complete cascading valorization.

Ruales et al. [110] provide an example of a completed sequential route. A biostimulant fraction was recovered from raceway-grown *Scenedesmus* biomass, and the residual material was anaerobically digested. Extraction increased methane yield by approximately 20%, from 244 to 293 mL CH_4 g^{-1} volatile solids, and improved digestion kinetics. Thus, biostimulant recovery was compatible with subsequent methane production.

A practical cascade can be conceptualized as algal biomass → high-value fraction(s) → phycobiostimulant-active fraction or residue → biomaterials or bioenergy → nutrient recovery. Because each upstream operation alters residual biomass, functionality, and value, stage-specific evidence is necessary to establish continued valorization rather than assuming it from process integration alone.

9.2. Waste-Derived Feedstocks and Resource Recovery

Wastewater treatment can be integrated with microbial resource recovery and circular biorefinery concepts [111]. Engineered microbial consortia can enhance degradation of recalcitrant xenobiotics and resilience of wastewater-treatment systems, supporting their integration into circular resource-recovery strategies [112]. Wastewater-grown microalgae and microalgae–microbe systems can couple nutrient removal with biomass generation for agricultural valorization [113]. Morillas-España et al. [114] operated an 80 m^2 raceway containing an indigenous microalga–bacteria consortium grown on urban wastewater; N assimilation into biomass accounted for 60–95% of measured N removal, while P was also removed. The treated effluent was used for hydroponic zucchini production, and the concentrated algal biomass was evaluated in plant bioassays, showing biostimulant potential through its effects on root development and chlorophyll retention [114].

Arashiro et al. [105] compared microalgal systems based on urban wastewater, food-industry wastewater, and conventional growth medium while incorporating recovery of pigments, biofertilizer, and biogas. Wastewater-based cultivation reduced several environmental burdens relative to conventional medium production, with food-industry

wastewater showing the most favorable overall profile [105]. However, these benefits remain feedstock- and process-specific.

Stranded macroalgae provide another secondary resource, but provenance is less controlled. Across six locations along the Mexican Caribbean, stranded biomass differed in abundance and species composition, and carbon content, ash-associated metals, particularly Fe and As, and isotopic composition varied spatially [115]. This heterogeneity and site-dependent contaminant load complicate feedstock specifications and quality control. Therefore, waste-derived or stranded biomass should be treated as a secondary feedstock requiring qualification rather than an inherently suitable agricultural input.

9.3. Safety as a Gate for Circular Reuse

Algae can concentrate undesirable compounds while recovering nutrients. Wastewater-derived biomass may contain heavy metals, pathogens, and contaminants of emerging concern (CECs), depending on source and cultivation conditions. Cyanobacterial feedstocks require additional strain- and source-specific safety assessments because some taxa produce cyanotoxins, such as microcystins or cylindrospermopsin, which can exert phytotoxic effects and accumulate in plant tissues, including edible organs [116].

For wastewater-grown microalgae or mixed microalgae–bacteria consortia, antibiotic-resistant bacteria, antibiotic-resistance genes (ARGs), and mobile genetic elements are additional concerns. *Chlorella vulgaris*–wastewater systems showed that treated wastewater increased ARGs and mobile genetic elements in the co-culture system, indicating potential resistance dissemination [117]. Therefore, cyanotoxin and ARG screening are relevant when the biomass source or cultivation history indicates risk.

Álvarez-González et al. [103] analyzed municipal-wastewater-grown microalgae intended for agricultural use for pathogens, metals, and 29 CECs. Twenty-five compounds were detected in wastewater, but only three in biomass. Most measured contaminants complied with applicable fertilizing-product thresholds, although Cd exceeded a limit. Contaminants detected in harvested lettuce were also present in controls, preventing specific attribution to microalgal treatment. Safety assessment is therefore feedstock- and exposure-pathway-specific rather than determined by wastewater origin alone.

The safety gate requires source traceability, contaminant characterization, and microbiological assessment before agricultural application. Analytical requirements vary with feedstock because municipal, industrial, and agricultural wastewaters and stranded marine biomass can present distinct contaminant profiles.

9.4. Sustainability Requires System-Level Verification

Life cycle assessment (LCA) and techno-economic assessment (TEA) provide system-level evaluation of circularity claims beyond feedstock origin. Arias et al. [106] compared macroalgal biostimulant production scenarios based on steam autoclaving, boiling, and supercritical fluid extraction. These routes differed in terms of energy and material demand, environmental impacts, and minimum selling price. Supercritical extraction showed the greatest environmental burden in most categories, largely because of energy and CO_2 requirements, whereas process optimization modified environmental and economic outcomes [106].

Harvesting and logistics are additional determinants of system performance. Rojo et al. [107] found that membrane harvesting reduced environmental impacts by approximately 30% relative to centrifugation in the evaluated scenarios. However, the transportation distance was influential in altering the preferred harvesting configuration.

In multiproduct biorefineries, environmental performance depends on how burdens and credits are allocated among phycobiostimulants, higher-value co-products, recovered

energy, and residual materials [105–107]. LCA outcomes remain conditional on the functional unit, system boundary, and allocation procedure rather than representing an intrinsic sustainability score.

10. Future Perspectives: Phycobiostimulants 2035—From Empirical Extracts to Precision Biological Inputs

This section presents prospective research directions rather than capabilities demonstrated for phycobiostimulants. The next phase of development can be organized around three priorities: (i) function-directed discovery linked to biological potency, (ii) mechanism-informed products with experimentally defined response domains, and (iii) predictive application supported by external validation and quantified uncertainty. These priorities represent increasing levels of evidence rather than formal regulatory categories. Chemical characterization alone does not constitute precision design unless the functional target, responsive biological context, and operating conditions are demonstrated.

Development differs among countries according to biomass resources, industrial experience, and research priorities. In Canada, *A. nodosum*-based products have a commercial and research base, with attention to composition and mechanisms of action [118]. In India, development is linked to commercial cultivation of *Kappaphycus alvarezii* and biomass and sap for agricultural applications, with efforts addressing scale-up, seed supply, biosecurity, and value-added utilization [119]. Australia has an established seaweed-extract industry and field-research base, particularly for preparations derived from *Durvillaea potatorum* and *A. nodosum*, with emphasis on stress resilience and optimization across crops and environments [120]. In China, research emphasizes microalgae and cyanobacteria as agricultural inputs, including their integration with nutrient recycling and circular-bioeconomy approaches [121].

10.1. Function-Directed Discovery

The first priority is function-directed selection of algal strains for specific agricultural functions rather than biomass productivity alone. The PhenoSelect platform integrates robotics, spectroscopy, fluorometry, flow cytometry, and data analysis to profile five microalgal species across 96 environmental and chemical conditions and quantify growth, photosynthetic performance, and cell size [122]. Yao et al. [123] developed a label-free mass-cytometry platform for rapid single-cell metabolite analysis, revealing metabolic heterogeneity masked by bulk measurements.

In phycobiostimulant discovery, algal phenotypes and metabolic fingerprints gain functional relevance when linked to standardized plant potency assays. Seven microalgal and cyanobacterial strains applied in a comparable suspension format had significantly different effects on cucumber germination kinetics, root growth, and seedling biomass [14]. Thus, function-directed discovery integrates algal phenotyping, chemical fingerprinting, and plant potency screening to identify strains with reproducible and traceable activities in a systematic manner.

The same principle applies to bioactivity-guided fractionation. Crude extracts can be separated, screened against a predefined potency assay, and active fractions characterized using metabolomic, proteomic, and structural approaches. Fraction-recombination experiments are important because activity may depend on interactions among components rather than a single molecule. *A. nodosum* studies support this concept: a fractionated lipophilic preparation was linked to molecular and physiological responses [39], whereas the reconstruction of major extract constituents showed that no single tested component reproduced the whole-extract response [51]. Therefore, the objective is to identify a reproducible chemical or functional fingerprint associated with biological potency.

10.2. Mechanism-Informed and Context-Specific Products

The second priority is to resolve genotype- and environment-dependent differences in product performance. A defatted *Chlorella* biomass extract tested across three barley genotypes and three salinity levels produced significant genotype × treatment interactions for grain weight and photosynthetic traits, together with genotype- and salinity-dependent nutrient responses [124]. Significant salinity × genotype × treatment interactions for physiological and nutrient traits support experimentally defined response domains across genotypes and environments, rather than broad crop-level claims.

Microbiome-aware formulations add to the specificity. They may recruit resident microorganisms or incorporate selected microbial partners, but introduced organisms must establish and remain functional within existing communities despite host and environmental variation. Copeland et al. [125] identified invasion, persistence, multi-stressor trade-offs, host effects, community instability, and delivery strategy as considerations for agricultural microbiome designs. Demonstrating functional complementarity and ecological compatibility therefore provides a stronger basis for algal–microbial consortium design than labeling individual components "beneficial."

Function-specific delivery systems may also be relevant. Matthews et al. [126] demonstrated a nano-encapsulated microbial–botanical biostimulant in chili, with changes in growth, yield, and fruit quality. However, because a composition-matched non-encapsulated control was unavailable, the contribution of encapsulation could not be resolved. Controlled-release or encapsulated formulations are justified only when direct comparisons demonstrate improvements in stability, bioavailability, delivery precision, or potency.

10.3. From Precision Products to Predictive Application

The final step involves linking product identity to crop status and environment. Functional phenotyping provides non-destructive, time-resolved measurements of plant structural and physiological traits relevant to precision agriculture [127]. Biostimulant-specific phenotyping frameworks integrate imaging, physiological sensing, omics and quantitative analyses during product development [101]. For phycobiostimulants, these approaches can provide dynamic biomarkers of responsiveness beyond terminal biomass and yields.

Machine learning can integrate product fingerprints, genotypes, soil characteristics, weather, developmental stages, application history, and phenotypic measurements. A phycobiostimulant-specific example was reported by Tanur Erkoyuncu et al. [128], who applied artificial neural network models to durum wheat seedlings treated with an *A. nodosum*-based seaweed extract under different salinity levels. The models predicted several physiological, biochemical, and nutrient traits with R^2 values above 0.90 and were implemented in an R Shiny application for real-time prediction. However, the experiment was conducted under controlled conditions and did not establish transferability across independent field environments. Sharifi et al. [129] predicted agronomic responses in rainfed wheat receiving seed priming and foliar micronutrient treatments. However, it was neither phycobiostimulant-specific nor designed to test cross-environment transferability.

Machine learning is an enabling tool rather than a substitute for standardized experimentation. Predictive performance within a development dataset does not establish transferability to a new site, season, or genotype. Agricultural modeling studies have shown that random internal validation can substantially overestimate performance relative to spatially independent external validation [130]. Transferability is tested using leave-location-out, leave-year-out, or prospective external validation. Predictive phycobiostimulant models, therefore, require datasets linking standardized product identity, genotype, environmental, and management variables, and agronomic response.

Third, matching a chemically and biologically specified phycobiostimulant with a responsive genotype, developmental stage, and environment while quantifying the probability of benefit is important. This requires interoperable product characterization, biological or physiological biomarkers, standardized environmental metadata, and multi-location and multi-year validation. Robust prediction also requires retention of weak, neutral, and negative responses to ensure that both response and non-response domains are represented. Precision phycobiostimulation moves from retrospective explanation of efficacy to prospective treatment selection based on externally validated predictions and quantified uncertainty.

11. Conclusions

Phycobiostimulant research has progressed beyond demonstrating that algal-derived products enhance plant growth and stress tolerance. The central challenge is to explain and control variability in agronomic responses among similar products. The proposed BPCME framework integrates biomass provenance, processing, chemical composition, biological mechanisms, and agronomic efficacy into a traceable continuum. Species or strain identity alone does not define a phycobiostimulant; cultivation conditions, harvest state, extraction, fractionation, and formulation collectively determine the chemical profile delivered to plants. Biological activity involves modulation of endogenous regulatory networks, including hormone crosstalk, redox regulation, metabolic reprogramming, and rhizosphere interactions. However, many proposed mechanisms remain associative rather than causally validated.

Predictable field performance requires control of product variability, as well as defined genotype, environment, and management contexts. The proposed QbD framework links candidate critical biomass and process attributes to chemical fingerprints, biological potency, and batch equivalence. Cascading biorefineries can improve resource utilization, provided they satisfy safety, traceability, and life-cycle criteria. Therefore, progress depends on connecting defined biomass and processing histories with reproducible chemical fingerprints, validated biological responses, batch-level potency, and reliable agronomic performance. These links span independent batches, genotypes, environments, and production systems.

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