WP2.1 – Microalgae as Biostimulants and Biopesticides

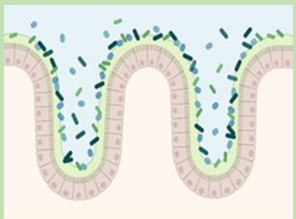
Use of Microalgae in Crop Production

Study of the Current State of Knowledge and Market Research

Reporting period 01.01.2025 – 31.12.2025

LP (MBU)



Bio2AgroFood

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1. SUMMARY

English summary

Microalgae and cyanobacteria are an arising category of natural biostimulants that improve plant growth, crop productivity, and tolerance to both biotic and abiotic stresses. These photosynthetic microorganisms are rich sources of primary metabolites—such as proteins, lipids, and carbohydrates—as well as diverse bioactive molecules. The bioactivity and bioavailability of microalgae were investigated using well-established *in vitro* model systems, with a particular focus on their functional potential beyond nutritional applications. In agriculture, they are recognized as a vast and renewable resource for biopesticides and crop protection agents. They produce a wide array of bioactive metabolites, including alkaloids, terpenoids, peptides, polysaccharides, and growth regulators showing potent antibacterial, antiviral, antifungal, nematocidal, and insecticidal properties. The microalgae-based solutions have strong potential of for environmentally friendly and sustainable agriculture.

Český souhrn

Mikrořasy a sinice představují vznikající kategorii přírodních biostimulantů, které zlepšují růst rostlin, výnos plodin a jejich toleranci vůči biotickým i abiotickým stresům. Tyto fotosyntetické mikroorganismy jsou bohatým zdrojem primárních metabolitů, jako jsou bílkoviny, lipidy a sacharidy, a také rozmanitých bioaktivních látek. Bioaktivita a biologická dostupnost mikrořas byly zkoumány pomocí dobře zavedených in vitro modelových systémů, se zvláštním zaměřením na jejich funkční potenciál přesahující nutriční využití. V zemědělství jsou považovány za rozsáhlý a obnovitelný zdroj biopesticidů a prostředků na ochranu rostlin. Produkují široké spektrum bioaktivních metabolitů, včetně alkaloidů, terpenoidů, peptidů, polysacharidů, a růstových hormonů, které vykazují silné antibakteriální, antivirové, antifungální, nematocidní a insekticidní účinky. Řešení založená na mikrořasách mají vysoký potenciál pro environmentálně šetrné a udržitelné zemědělství.

Německý souhrn

Mikroalgen und Cyanobakterien stellen eine aufkommende Kategorie natürlicher Biostimulanzien dar, die das Pflanzenwachstum, die Ernteerträge sowie die Toleranz der Pflanzen gegenüber biotischen und abiotischen Stressfaktoren verbessern. Diese photosynthetischen Mikroorganismen sind eine reichhaltige Quelle primärer Metaboliten wie Proteine, Lipide und Kohlenhydrate sowie einer Vielzahl bioaktiver Substanzen. Die Bioaktivität und Bioverfügbarkeit von Mikroalgen wurde mithilfe gut etablierter in-vitro-Modellsysteme untersucht, wobei der Schwerpunkt auf ihrem funktionellen Potenzial über die rein ernährungsphysiologische Nutzung hinaus lag. In der Landwirtschaft gelten sie als umfangreiche und erneuerbare Quelle für Biopestizide und Pflanzenschutzmittel. Sie produzieren ein breites Spektrum bioaktiver Metaboliten, darunter Alkaloide, Terpenoide, Peptide, Polysaccharide und Phytohormone, die ausgeprägte antibakterielle, antivirale, antifungale, nematozide und insektizide Wirkungen aufweisen. Auf Mikroalgen basierende Lösungen besitzen ein hohes Potenzial für eine umweltfreundliche und nachhaltige Landwirtschaft.

2. LEGISLATION FRAMEWORK

The legislative landscape for biostimulants and biofertilisers has undergone significant changes recently, particularly in Europe, as authorities move to distinguish these products from traditional fertilizers and pesticides. Until recently, the biostimulant market was governed by a fragmented mix of national laws, leading to inconsistent naming and market barriers. The primary regulatory shift occurred with the implementation of Regulation (EU) 2019/1009 (The Fertilising Products Regulation – FPR), which became fully applicable in July 2022.

A plant biostimulant is defined as an EU fertilising product that stimulates plant nutrition processes independently of its nutrient content. Its sole purpose is to improve nutrient use efficiency, tolerance to abiotic stress, quality traits, or the availability of confined nutrients in the soil or rhizosphere.

The regulation distinguishes between two categories of plant biostimulants – Microbial plant biostimulants, which consisting of a micro-organism or a consortium of micro-organisms and Non-microbial plant biostimulants, which covers plants, parts of plants, or vegetal extracts and microalgal extracts.

According to European legislation, algae (including microalgae) must meet specific safety and processing criteria to be marketed as biostimulants:

- **Permitted Processing**: Only certain processes are allowed for preparing extracts, including centrifugation, grinding, milling, drying, freeze-drying, and extraction with water or supercritical CO_2.
- **Contaminant Thresholds**: Regulation (EU) 2019/1009 sets strict limits on heavy metals and pathogens for non-microbial biostimulants (CFP 6-B):

◦ Cadmium (Cd): 1.5 mg/kg.

◦ Lead (Pb): 120 mg/kg.

◦ Mercury (Hg): 1.0 mg/kg.

◦ Pathogens: Salmonella spp. must be absent in 25 g/25mL, and *E. coli* or *Enterococcaceae* must not exceed 1000 CFU in 1 g/1mL.

Notably, biostimulants extracted specifically from cyanobacteria are not yet included in the current European legislation and cannot be legally commercialized as biostimulants in the EU at this time.

Marketers have two pathways to enter the European market:

- National Regulation, where the product is marketable in one Member State, and access to others is sought through the principle of mutual recognition (Regulation (EU) 2019/515)
- EU Harmonized Process

Once a product receives an EU-type certificate and the CE mark, it gains access to the entire EU market. The legislative and regulatory landscape for using microalgae and cyanobacteria as biopesticides is defined by a distinction between product categories, voluntary technical standards, and safety assessments regarding toxins and biosafety.

Currently, the commercialization of microalgae-based products in agriculture is primarily in the early stages. Due to the high costs associated with biomass production and the rigorous regulatory requirements for pesticide registration, many microalgae-derived products are currently registered and marketed as plant biostimulants rather than biopesticides. Biostimulants are generally regulated based on their ability to improve nutrient efficiency and stress tolerance, whereas biopesticides require more extensive data to prove specific efficacy against pests.

The technical framework for evaluating the effectiveness of these substances relies on standards developed by organizations such as the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). These organizations provide voluntary consensus standards for broth microdilution and other testing methods to ensure results are reproducible and comparable across laboratories. However, it is important to note that conformance to these standards does not relieve the user or manufacturer of responsibility for compliance with applicable national or regional regulations.

The Gap in Interpretive Criteria showsa significant hurdle for the legislative approval of new biopesticides is the lack of clinical interpretative breakpoints for many algae-pathogen combinations.

Regulatory oversight for cyanobacteria-based products must strictly account for cyanotoxins.

Current research is aimed at providing the MIC-outcome correlations necessary for the future establishment of robust clinical breakpoints, which will eventually facilitate clearer legislative pathways for these biological agents. Optimization of strains that balance high biomass productivity with consistent bioactivity remains a prerequisite for making these products economically and legally viable compared to synthetic alternatives

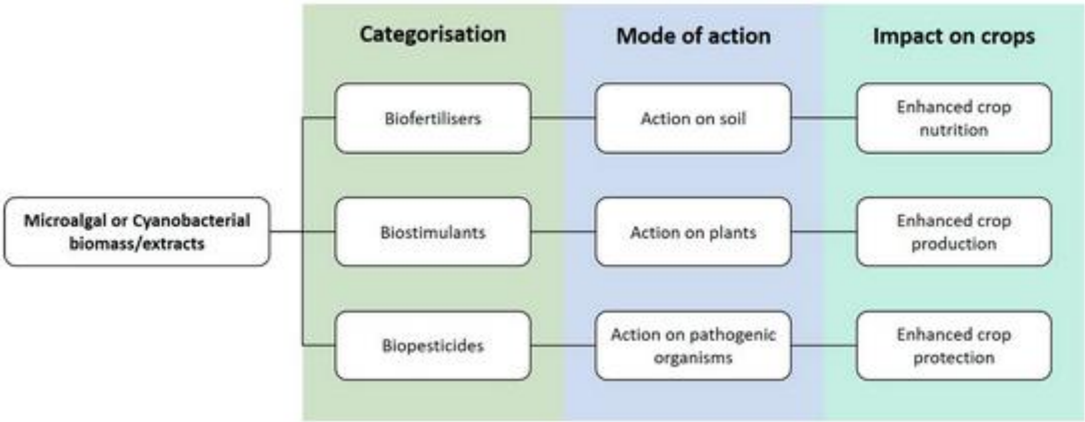
3. USE OF MICROALGAE IN AGRICULTURE

Using microalgae in agriculture is an emerging strategy to enhance agricultural sustainability by providing renewable alternatives to synthetic fertilizers and pesticides. Microalgae and cyanobacteria are microscopic photosynthetic organisms that produce high-value metabolites, including proteins, phytohormones, polysaccharides, and antioxidants, which benefit both plants and soil.

They exhibit potent antimicrobial, insecticidal, and herbicidal activities. These organisms serve as a sustainable alternative to synthetic pesticides because they are readily biodegradable, have low residuality, and display high specificity toward target pests.

They can be divided into 3 class: Biofertilisers, Biostimulants and Biopestiides.

Biofertilisers focus on improving soil chemical and biological properties and restoring soil fertility. Biopesticides are used to manage pests in agricultural practices, unlike biostimulants, which primarily enhance physiological processes and stress tolerance regardless of nutrient content.



(Source: Gonçalves et al., 2021)

3.1 Biofertilisers

Biofertilisers are biological products containing living microorganisms such as bacteria, fungi, and microalgae. After applicationton of seeds, plant surfaces, or soil, promote growth by increasing the supply or availability of primary nutrients to the host plant.

Biofertilisers mobilize nutrient availability in soils by colonising the rhizosphere, rhizoplane or roots interior.

They can be divided into 6 groups:

1) **Nitrogen fixation**

   Nitrogen-fixing biofertilisers, primarily composed of cyanobacteria such as *Anabaena* and *Nostoc*, utilize the nitrogenase enzyme complex to transform atmospheric N_2 into bioavailable ammonia for easy plant assimilation.

2) **Potassium mobilising and solubilising**

   Potassium-solubilising biofertilisers, including specific *Bacillus* strains and various microalgae, enhance plant nutrition by excreting organic acids that directly dissolve rock minerals like micas or chelate associated silicon ions. These biological agents promote sustainable growth by acting as slow-release nutrient sources and helping to re-establish cellular ion homeostasis under environmental stressors such as salinity.

3) **Phosphate mobilising and solubilising**

   Microorganisms produce extracellular enzymes like phosphatases and phytases or release organic acids to transform insoluble soil phosphorus into bioavailable forms for plants.

4) **Siderophore Production**

   Some microorganisms secrete siderophores, which are molecules that sequester iron from the soil, making it available to the plant.

5) **Soil Structure Improvement**

   Many microalgae and bacteria release exopolysaccharides (EPS), which act as soil conditioners by improving aggregation, porosity, and water-retention capacity.

6) **Phytohormone Production**

   Biofertilisers often produce plant growth regulators like auxins, cytokinins, and gibberellins, which directly influence root development and cell division.

Key microorganisms of biofertilisers are Cyanobacteria and Green Microalgae. Cyanobacteria or Blue-green algae represent species like *Nostoc*, *Anabaena*, and *Aulosira*. They are widely valued for their ability to fix atmospheric nitrogen (N_2), converting it into forms easily assimilated by plants. Green microalgae, as Common species, include *Chlorella vulgaris*, *Scenedesmus* sp., and *Arthrospira* (Spirulina), which contain significant amounts of macronutrients (N, P, K) and micronutrients.

Biofertilisers can be applied using several techniques depending on the crop and the intended outcome. These include seed treatment, such as seed priming, coating, or dipping in microbial suspensions, which gives plants a head starts during germination. Soil application involves the direct incorporation of live cultures or dried biomass in the form of powders or pellets into the soil or substrate, often via soil drenching. In the case of foliar application, extracts or supernatants are sprayed onto leaves to enable rapid nutrient absorption through stomata and cuticle pores. In addition to their versatile application methods, biofertilisers are considered environmentally friendly alternatives to synthetic fertilisers because they reduce nutrient leaching and prevent nitrate water pollution. Moreover, the production of microalgal biofertilisers can be integrated into a circular economy by using wastewater and industrial by-products as nutrient sources, which lowers production costs and helps mitigate greenhouse gas emissions.

3.2 Biostimulants

Biostimulants promote crops' productivity by acting directly on the plant. These compounds are responsible for improving respiration, photosynthetic activity, nucleic acid synthesis and ion uptake, enhancing plants' metabolism and, hence, plants' growth. The physiological and biochemical mechanisms through which microalgae function as biostimulants and biofertilisers are multifaceted, involving the production of signaling molecules, the enhancement of metabolic pathways, and the mitigation of environmental stress.

3.2.1 Phytohormones and Plant Growth Regulation

Phytohormones and hormone-like signaling molecules produced by microalgae and cyanobacteria act as chemical messengers to regulate plant growth, development, and stress responses. These biostimulants are effective in small concentrations and can influence multiple physiological processes, including cell division, nutrient mobilization, and morphogenesis (Table 1).

The major classes of phytohormones include the following:

· **Auxins** (e.g., IAA - Indole-3-acetic acid, IAM - Indole-3-acetamide, IBA - Indole-3-butyric acid): Their role is promoting cell division and elongation by activating plasma membrane H^+-ATPase. They are essential for root initiation and development.

· **Cytokinins** (e.g., Zeatin, Kinetin): They stimulate cell division, promote shoot development, and delay leaf senescence. In microalgae, *cis*-zeatin is often the predominant form.

· **Gibberellins** (GAs): These hormones regulate stem elongation, seed germination, and floral organ development. They act synergistically with auxins but can act antagonistically with abscisic acid.

· **Abscisic Acid** (ABA): Primarily known as a growth inhibitor, ABA regulates seed maturation and induces stomatal closure to manage water loss under abiotic stress.

· **Ethylene** (ET): It regulates fruit ripening and senescence. Some microalgae produce ACC (L-aminocyclopropane-1-carboxylate) deaminase, an enzyme that degrades the ethylene precursor ACC, thereby reducing inhibitory ethylene levels and protecting plants from stress-induced growth inhibition.

· **Additional signalling molecules include**:

◦ **Brassinosteroids** (BRs): Act as growth promoters and protect against abiotic stresses like salinity and temperature extremes.

◦ **Jasmonic** (JA) and **Salicylic Acids** (SA): Central to regulating plant defense against pathogens and insects.

◦ **Polyamines** (e.g., Spermine): Function similarly to hormones by stimulating cell division and increasing metabolic activity.

Mechanisms of Action showed that microalgae biostimulants can deliver these hormones directly to the plant or rhizosphere, or they can act as elicitors that trigger the plant's endogenous synthesis of its own hormones. A specific molecular mechanism identified in Arabidopsis involves microalgae inducing the expression of the IAA5 gene. This triggers a transcriptional cascade where IAA5 suppresses the WRKY63 transcription factor, leading to the activation of MYB28 and MYB29, which increases glucosinolate levels and enhances drought tolerance by facilitating stomatal closure.

3.2.2 Exopolysaccharides (EPS) and Defence-Signaling Activation

The role of EPS in triggering plant defense systems is a critical aspect of their function as microalgae-based biostimulants. These complex carbohydrate polymers serve as signaling molecules that initiate a cascade of physiological and biochemical reactions within the plant.

Microalgal and cyanobacterial polysaccharides are perceived by plant membrane receptors as microbial-associated molecular patterns or pathogen-associated molecular patterns. These receptors, such as leucine-rich repeat receptors and toll-like receptors located in the plasma membrane, recognize the EPS as chemical cues of potential microbial presence. This recognition immediately triggers a series of primary signaling events, including a rapid change in cellular calcium levels that acts as a secondary messenger anda an "oxidative burst" resulting in the production of reactive oxygen species (ROS), which function as signaling agents to activate further defense genes.

The initial signals lead to the upregulation of key metabolic pathways that produce defense-related metabolites. It induces process activation ofPhenylalanine ammonia-lyase (PAL) and Lipoxygenase (LOX).

PAL is a key element in the lignification process and the synthesis of phenolic substances and phytoalexins (toxic properties against pathogens) and LOX triggers

synthesis of oxylipins, which are precursors to defense hormones like Jasmonic Acid (JA). EPS treatment modulates the activity of Salicylic Acid (SA) and Jasmonic Acid (JA) signaling pathways, which are responsible for the plant's overall resistance to biotrophic and necrotrophic pathogens.

Beyond biochemical signaling, EPS perception leads to physical changes that enhance tolerance to both biotic and abiotic stressors (Cuticular Wax Synthesis, Hydrophobic Barrier, Lignification).

Table 1: Summary of phytohormone and key algal sources

Phytohormones	Microalgal/Cyanobacterial Sources	Physiological action
Auxins	*Chlorella, Coenoochloris, Acutodesmus and Scenedesmus, Synechocystis, Chroococcidiopsis, Anabaena, Phormidium, Oscillatoria, Nostoc, Chlorogloeopsis*	Cell elongation, differentiation of phloem, apical dominance, tropism, initiation of root formation and abiotic stress tolerance
Cytokinins	Chlorella, Chlamydomonas, Desmococcus, Euglena, Myrmecia, Stigeoclonium, Mychonastes, Scenedesmus, Nannochloropsis, Nautococcus, Protococcus, Arthronema, Synechocystis, Chroococcidiopsis, Anabaena, Phormidium, Oscillatoria, Calothrix, Chlorogloeopsis, Rhodospirillum	Control cell division, promotion of protein and chlorophyll synthesis, development of chloroplast, vascular tissue, bud, fruit, flower, leaf blade; shoot growth, delaying senescence, higher abiotic stress tolerance
Gibberellins	*Scenedesmus, Stigeoclonium, Gyoerffyana, Myrmecia, Nautococcus, Chlorella, Scotiellopsis, Chlorococcum, Chlamydomonas, Nannochloropsis, Arthrospira, Anabaenopsis, Cylindrospermum, Phormidium*	Stem elongation, initiation of seed germination (via activation of α-amylase), floral organ development, protein biosynthesis, initiation of flowering
Brassinosteroids	Klebsormidium	Controls division, elongation and differentiation of vascular system, promotion of ethylene production, stress tolerance
Ethylene	*Chlorella, Scenedesmus Arthrospira, Synechococcus, Anabaena, Nostoc, Calothrix, Scytonema, Cylindrospermum*	Regulates development processes (fruit ripening, opening of flowers, cell division, cell elongation, senescence of vegetative tissues, biotic and abiotic stress tolerance)
Abscisic acid	*Scenedesmus, Chlorella, Dunaliella, Haematococcus, Chlamydomonas, Nannochloropsis, Synechococcus, nostocmuscorum, Trichormusvariabilis, Anabaena*	Induces stomatal closure, shoot growth inhibition, protein storage in seeds with seed dormancy

Jasmonic acid	*Chlorela, Scenedesmus, Dunaliella, Euglena, Gelidium*	Plant defence response regulation, synthesis of proteinase inhibitors, promotion of tuber formation and senescence
Salicylic acid	*Scenedesmus, Arthrospira*	Plant defence response regulation during pathogenesis
Polyamines	*Chlorella, Cyanidium, Euglena, Arthrospira*	Stress response regulation and metabolic processes such as cell proliferation, differentiation and growth

3.2.3 Antioxidants, Amino Acids, and Metabolities Enhancing Stress Tolerance

The use of microalgae and cyanobacteria as biostimulants involves a wide array of antioxidants, amino acids, and specialized metabolites that function collectively to enhance plant tolerance against environmental stressors such as drought and salinity (Table 2). The following classification can be distinguished:

A. Antioxidant Defense Systems

Abiotic stress induces the excessive accumulation of ROS, such as hydrogen peroxide and superoxide radicals, which cause oxidative damage to lipids, proteins, and DNA. Microalgae-based biostimulants mitigate this damage by activating both enzymatic and non-enzymatic defense mechanisms. In terms of Enzymatic Antioxidants, application of microalgae like *Chlorella vulgaris* significantly increases the activity of key enzymes including superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase. These enzymes coordinate to scavenge ROS and maintain cellular redox homeostasis. Conversely, Non-Enzymatic Antioxidants are pigments and phenolic compounds that act as powerful antioxidants. Carotenoids (e.g., β-carotene, lutein, and astaxanthin) protect the photosynthetic machinery from photodamage, while phenolic acids and flavonoids neutralize free radicals and chelate toxic metal ions.

B. Amino Acids and Polyamines

Amino acids serve as building blocks for proteins and precursors for defense-related signaling molecules. Proline is a critical amino acid that accumulates in response to salt and drought stress, where it functions as an osmoprotectant to maintain cell turgor and stabilize cell membranes. Glycine betaine similarly acts as an osmolyte, protecting Photosystem II and preventing chlorophyll degradation under stress. Tryptophan acts as a precursor for the growth hormone auxin and the defense signal salicylic acid, while arginine is the precursor for polyamines.

Molecules like spermine, spermidine, and putrescine are poly-cations involved in regulating the cell cycle and inhibiting senescence. Treatment with cyanobacterial hydrolysates has been shown to increase leaf spermine content by 64 %, leading to more robust growth.

C. Metabolites for Structural Adaptation

Microalgae biostimulants trigger the synthesis of specific lipophilic metabolites that strengthen the plant's physical barriers (Synthesis of Cuticular Wax – hydrophobic barrier, derivatives of Vitamin B3 – precursor for energy production and adaptation to stress).

Table 2: Microalgal and cyanobaterial metabolities with potential interest for agricluture

Metabolities	**Microalgal/Cyanobacterial Sources**	**Biological activity**
Phenolic compounds	*Botryococcus braunii; Chaetoceros calcitrans; Chlorella vulgaris; Isochrysis galbana; Isochrysis sp.; Neochloris oleoabundans; Odontella sinensis; Phaeodactylum tricornutum; Sacharina japonica; Skeletonema costatum; Tetraselmis suecica*	crop´s protection against biotic and abiotic stress conditions
Terpenoids	*Chornococcus hornemanni; Hypnea pannosa; Oscillatoria perornata; Planktothricoids raciborskii; Plocamium cornutum; Plocamium leptophyllum; Portieria hornemann; Pseudanabaena articulate; pseudanabaena* sp.; *Sphaerococcus coronopifolius; Synechocystis* sp.; *Thermosynechococcus elongatus*	Crop´s protection against bacteria, insects and other organisms; stimulation of preliminary growth and development of plants; attraction of pollination
Free fatty acids	*Anabaena; Chlorella; Dunaliella; Nannochloropsis; Porphyridium; Scenedesmus; Spirulina*	Crop´s protection against pathogens or other biotic and abiotic stress condition
Polysaccharides	*Aphanohtece; Arthrospira; Chlamydomonas; Chlorella; Cylindrotheca; Dunaliella; Navicula; Nostoc; Phaedodactylum; Porphyridium; Rhodella; Scytonema*	Improvement of soil quality, plant growth stimulation, crop´s protection against biotic and abiotic stress conditions
Carotenoids	*Chlorela protothecoides; chlorela pyrenoidosa; Chlorela zofingiensis; Dunaliella salina; Haematococces*	Soil bioremediation and fertilisation, crop´s protection against bacteria, insects and

	pluvalis; Muriellopsis sp.; Phaeodactylum tricornutum; Spirulina sp.	other biotic and abiotic stress conditions; fortification
Phytohormones	*Arthrospira; Chlamydomonas; Chlorella; Phormidium; Protococcus; Scenedesmus*	Plant growth stimulation; regulation of cellular activities in crops; crop´s response to stress conditions

3.2.4 Modulation of Photosynthesis and Pigment Biosynthesis

The modulation of photosynthesis and pigment biosynthesis is one of the most significant biostimulatory effects of microalgae and cyanobacteria-based biostimulants (MCBs) on crops. These effects occur through the direct stimulation of pigment production, protection of the photosynthetic apparatus under stress, and the upregulation of key metabolic enzymes.

Microalgae application consistently increases the concentration of chlorophyll a, chlorophyll b, and total carotenoids across various plant species. They are rich in L-glutamic acid, which serves as a metabolic precursor for the synthesis of chlorophyll via the aminolaevulinic acid pathway.

In broccoli plants under drought stress, foliar application of Chlorella vulgaris increased chlorophyll a by 6–60 % and total carotenoids by 26–114 %. Similarly, tomato plants treated with certain microalgal extracts showed chlorophyll b increases of up to 93 %. In "Red Russian" kale, treatment with microalgal culture supernatant triggered a 5.9-fold increase in anthocyanins, which are vital for photoprotection and plant defense.

MCBs influence the efficiency of the Calvin cycle and the stability of the light-harvesting complex.

Algal extracts have been shown to upregulate genes involved in carbon fixation, specifically increasing the synthesis and activity of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) and carbonic anhydrase. Metabolites such as glycine betaine and polyamines found in microalgae stabilize the structure of Photosystem II (PSII) and prevent its degradation during environmental stressors like high light, drought, or salinity. Application of MCBs improves stomatal conductance and the net photosynthetic rate (Pn), ensuring continued biomass accumulation even when water is limited.

MCBs have also effect on Stress Mitigation and Photoprotection. One of the primary roles of microalgae-derived pigments and antioxidants is to shield the photosynthetic system from oxidative damage.

Stress-induced ROS can lead to chlorophyll degradation (chlorosis). MCBs activate enzymatic defense systems, that neutralize these radicals, thereby maintaining the integrity of the photosynthetic membrane.Additionally

Algal carotenoids, such as *β*-carotene and lutein, act as lipophilic antioxidants that protect the chloroplast from photodamage and thermal stress. Notably, C-phycocyanin, a specialized pigment in cyanobacteria like *Arthrospira*, has been identified as a biostimulant that promotes leaf diameter expansion and increases flavonoid content in hydroponic systems.

Regarding hormonal and molecular signaling, bioactive molecules in MCBs act as chemical messengers to regulate the plant's internal photosynthetic priorities. Cytokinins are known to delay leaf senescence and maintain high photosynthetic activity by diminishing the negative effects of stress on the chloroplast. Algal polysaccharides (such as β-glucan) interact with receptors in the plant plasma membrane to induce signaling cascades that increase the synthesis of Rubisco and improve PS II activity. Finally, Eethylene may regulate photosynthesis and cell remodeling in response to abiotic stress.

3.2.5 Metabolomic Reprogramming

Metabolomic reprogramming (metabolic reconfiguration) refers to the biostimulant-induced redistribution and remodeling of plant metabolic pathways to improve growth, quality, and resilience against environmental stresses.

Based on the sources, the key components of this reprogramming include:

A. Lipid Remodeling and Barrier Construction

Microalgal biostimulants trigger a significant shift in lipid metabolism. They promote the synthesis of Very Long-Chain Fatty Acids and alkanes, which are essential components of cuticular wax. This creates a hydrophobic barrier on leaves that reduces unregulated water loss and inhibits pathogen invasion.

B. Coordinated Carbon (C) and Nitrogen (N) Metabolism

Reprogramming often involves the simultaneous regulation of C and N assimilation.

Biostimulants increase the activity of key enzymes like nitrate reductase and glutamine synthetase, leading to higher protein content and a redistribution of amino acids. Plants under biostimulation may show a decrease in certain sugars (like sucrose) as they are converted into energy or structural components to support rapid growth and "sink" organ development (e.g., larger onion bulbs or apple fruits).

C. Upregulation of Defense and Secondary Metabolites

Through metabolomic reprogramming, plants prioritize the production of protective compounds, such as glucosinolates, anthocyanins and antioxidants like flavonoids and phenolic acids.

D. Osmotic Adjustment and Antioxidant Defense

Under stress (salinity or drought), the plant’s metabolome is "reprogrammed" to accumulate osmoprotectants such as proline and glycine betaine. These solutes stabilize cell membranes and enzymes, while increased activity of scavenging enzymes neutralizes ROS to prevent cellular damage.

3.2.6 Biostimulant products from microalgae

Microalgae are used in various forms, such as powders, extracts or liquid concentrates. They are applied in agriculture to improve plant growth, stress resistance or protection against pathogens (Table 3).

Examples of products based on microalgae as biostimulants:

- Agribest Plus - solution of amino acids from microalgae formulated using Trietech© Technology, with prebiotic action
- AlgaE - biostimulants based on microalgae that improve plant growth, resistance to stress and increase yields ((Algatechnologies company)
- Algafert – microalgae complement to improve and enhance fruit and vegetable production, https://www.biorizon.es/en/products/biostimulants-y-bioenhancers/algafert/
- Algafert Eco - liquid biostimulant formulated with microalgae extract, P_2O_5 (0.15%), N (1.5%) and K_2O (3.4%), https://www.biorizon.es/en/products/biostimulants-y-bioenhancers/algafert-eco/
- Biofat 600 - a liquid biostimulant made from microalgae extracts with 5% N and 7% calcium (CaO), https://www.biorizon.es/en/products/biostimulants-y-bioenhancers/biofat-600/
- Biopower Eco - liquid biostimulant based on microalgae extract and 1.8% amide nitrogen (NH2), https://www.biorizon.es/en/products/biostimulants-y-bioenhancers/biopower-eco/
- ERANTHIS – product of Green Has Italia S.p.A from seaweeds *Ascophyllum nodosum*, *Laminaria digitata* and yeast extracts
- Chlorella vulgaris – microalgae extract is used as a biostimulant in agriculture to improve plant health and increase their resistance to stress
- GrowBetter - a commercial product based on microalgae that improves plant growth, disease resistance and promotes healthy root system development produced by AlgaEnergy company
- Phycocyanin - extract from microalga Spirulina, which has applications in agriculture as a biostimulant, improves photosynthesis and promotes growth
- TechSeaLab - both marine and terrestrial wide variety of fertilisers, Breton company (https://www.techsealab.com/en/agriculture)

Table 3: Biostimulatory effect of biomass on agronomically important plants

Microalgae Cyanobacteria	**Target plant**	**Observed improvement**
Anabaena strains	Rice	Increase germination, plant height and weights, shoot length, soil moisture and porosity
Arthrospira platensis	Lettuce	Increase in seedling growth and spermin content in leaves
Laurentia obtusa, Corallina elongata, Jania rubens	Maize	Increase in plant length, weight, number of leaves Improvement of plant nutritional value (P, N, K)

Chlorella protothecoides	Cucumber, barely, wheat, soybean, watercress	Increased germination, root elongation
Chlorella vulgaris	Cucumber wheat, soybean, watercress, tomato	Increased germination, root elongation
Chlorela vulgaris, Spirulina platensis	Maize	Increase in germination rate, plant yield, fresh and dry weights of whole plant, shoot length, in number of leaves
Dunaliella salina (extrapolysaccharides extracts)	Wheat	Increase in germination rate and seedlings growth Increase in root and coleoptiles height, in tolerance in salt stress
Gracilaria corticata, Enteromorpha flexuosa (aqueous extracts)	Maize, sunflower	Increase in shoot, root length, dry weight Improvement of plant nutritional value (photosynthetic pigment, carbohydrate, proteins and nutrient contents)
Tetradesmus obliquus	Wheat, watercress	Little improvement of germination
Tetradesmus obliquus	Cucumber, barely, wheat, soybean, watercress, tomato	Increased germination, root elongation
Spirulina platensis (total polysaccharides extract)	Tomato, pepper	Increase in plant size, root weight, size and numbers of nodes
Synechoscistis sp.	Cucumber, barely, wheat, soybean, watercress	Increased germination, root elongation
Stoechospermum marginatum (liquid extracts)	Eggplant	Increase in shoot and root length, weight, leaf area Improvement of leaves nutritional value (moisture, photosynthetic pigment, protein, amino acids; reducting sugar and ascorbic acid contents)
Ulva lactuca, Jania rubens	Spinach	Increase in plant yield and height Improvement of plant nutritional value (chlorophyll, nitrogen contents)

3.3 Biopesticides

Microalgae and cyanobacteria are recognized as a wide-ranging and sustainable resource for biopesticides, producing a of extensive bioactive metabolites. These substances show strong antibacterial, antiviral, antifungal, nematocidal, and insecticidal activities. They operate via multiple mechanisms, including the inhibition of photosynthesis (acting as herbicides), neurotoxicity (acting as insecticides), and the induction of plant defense responses**.**

Biopesticides, derived from natural organisms, offer a sustainable alternative due to their high biodegradability, lower residuality, and better specificity toward non-target organisms. Among the most promising sources are microalgae and cyanobacteria. These photosynthetic microorganisms inhabit diverse and extreme ecological niches, having evolved a vast array of secondary metabolites—such as alkaloids, peptides, and polysaccharides—to survive competitive pressures.

Recent research indicates that approximately 45% of tested microalgal species demonstrate mycelial growth inhibitory activity against at least one fungal pathogen.

3.3.1 Mechanisms of action

Algal metabolites function through diverse biological pathways to control pests and pathogens:

• **Inhibition of Photosynthesis:** Many allelopathic substances, such as cyanobacterin isolated from *Scytonema hofmanni*, specifically interrupt photosynthetic electron transport in photosystem II. This mechanism is highly effective against weeds and competing phototrophic organisms.

• **Induction of Plant Defense Responses:** Algal polysaccharides like laminarin, ulvan, and carrageenans act as elicitors. They are recognized by plant cell receptors, triggering defense pathways such as salicylic acid,, jasmonic acid, and ethylene signaling. This leads to an "oxidative burst" and the production of pathogenesis-related proteins. They improve the plant's natural immune responses

• **Quorum Sensing Inhibition:** Certain algae produce halogenated furanones that mimic signaling molecules used by bacteria to colonize hosts, effectively blocking their communication and reducing virulence.

• **Neurotoxicity:** Halogenated monoterpenes and sterols found in species like *Plocamium telfairiae* and *Prasiola crispa* exhibit insecticidal activity by targeting picrotoxinin and octopaminergic-cholinergic receptors in insects.

• **Antimetabolites:** Compounds like 7-deoxysedoheptulose block producing essential aromatic amino acids in pathogens without affecting animals.

3.3.2 Bioactive Compounds

Studies have identified numerous strains exhibiting strong antagonistic activity against phytopathogenic fungi and bacteria (Table 4). They transitioned from basic extract screening to the identification of specific active compounds using advanced tools like high-performance countercurrent chromatography, MS/MS-based molecular networking, and dereplication. These technologies allow to discover of novel molecules with elimination of known redundant compounds. Notable success has been found in the following areas

• **Antifungal Activity:** Strains from the order Nostocales are particularly metabolically versatile. Specifically, *Tolypothrix tenuis* MACC-205 and *Trichormus variabilis* MACC-304 have shown broad-spectrum activity against multiple pathogens, including *Botrytis cinerea* and *Fusarium graminearum*. Additionally, muscotoxins (cyclic lipopeptides) from *Desmonostoc muscorum* exhibit strong activity against *Alternaria alternata* and *Aspergillus fumigatus*.

• **Biocontrol of Soilborne Pathogens:** The green microalgal genus *Desmodesmus*, particularly *D. subspicatus*, isolated from plant rhizospheres, has emerged as a robust candidate, demonstrating broad-spectrum inhibition of soilborne pathogens. *Chlorella fusca* extracts have also been used to suppress *Fusarium* wilt in strawberries.

• **Defense Signaling:** Extracts from *Chlorella vulgaris* and *C. sorokiniana* have been shown to trigger innate plant defenses by increasing enzymes like -1,3-glucanase and phenylalanine ammonia lyase in host plants like tomatoes.

• **Photosynthesis Inhibition:** Compounds like cyanobacterin isolated from *Scytonema hofmanni* act as natural herbicides by specifically interrupting photosynthetic electron transport in competitors.

3.3.3 Commercial Products and Market Status

The commercialization of microalgae-derived biopesticides is currently in the early stages. While lab results are promising, high downstream production costs—such as harvesting and processing biomass—remain a limitation to competing with synthetic alternatives.

Most available products are currently registered as plant biostimulants rather than pure biopesticides, though they often provide protective benefits. Notable commercial formulations include:

• **Kelpak** and **Maxicrop:** Derived from *Ascophyllum nodosum* and *Ecklonia maxima*, these are used to manage soil nematodes and red spider mites.

• **Algaefol:** A brown seaweed-based product used against various root-knot nematodes in crops like citrus and tomato.

• **OSMO:** An algal product designed to alter the sensory perception of roots by nematodes, preventing infection

Table 4: Microalgae and cyanobacteria extract exhibited biopesticidal activity against agronomically important plant pathogens

Mode of action	Microalgae Cyanobacteria extract	Target plant	Observed improvement
Protection against biotic factors – fungicidal activity	*Laminaria digitata*	gapevine	Fungicidal activity against *Botrytis cinerea, Plasmopara viticola*
	Species of *Oscillatoria, Anabaena, Nostoc, Nodularia, Calothrix*	pepper	Fung. act. against *Alternaria alternate, Botrytis cinerea*
	Oscilatoria chlorina	tomato	Fung. act. against *Meloidogyne arenaria*; improve plant growth
	Ulva armoricana (extracts)	Bean, grapevine, cucumber	Fung. act. against *Erysiphe polygoni, Erysiphe necator, Sphareotheca fuliginea*; reduced foliar disease
	Anabaena variabilis and *Anabaena oscillarioides*	tomato	Reduction in fungal disease severity; improved plant growth
	Spatoglossum variabile, Stokeyia indica, Melanothamnus afaqhusainii	Watermelon, eggplant	Fung. act. against the root rotting fungi, *Fusarium solani, Fusarium oxysporum, Macrophomina phaseolina* Antinematodal act. against *Meloidogyne* spp., Improved plant growth
Protection against biotic factors – antibacterial activity	*Sargasus wightii*	rice	Antibacterial activity against *Xanthomonas oryzae*
	Cystoseria myriophylloides and *Fucus spiralis*	tomato	Ant. act. against *Agrobacterium tumefaciens*
	Chlorella fusca	Arabidopsis	*Pseudomonas syringae*
Chewing insects	*Scytonema*	Cotton	*Helicoverpa armigera, Helios larvae, Sylepta derogata*
Another improvement	*Anabaena laxa* and *Calothrix elenkinii*	Coriander, cumin, fennel	Increase in fungicidal activity, germination rates, in shoot and root length, plant dry weight, activity of β-1,3 endonuclease in shoots and roots
	Anabaena torulosa, Anabaena laxa, Anabaena azollae, Anabaena	wheat	Increase in nitrogen fixation, hydrolytic and defence enzymes, fresh and dry weight of plant

	oscillarioides, Calothrix sp.		
	Calothrix elenkinii	rice	Increase in nitrogen activity and IAA production, hydrolytic and defence enzymes, plant growth
	Chlorella vulgaris, Ch. sorokiniana, Chlamydomonas reinhardtii – polysach. extract	tomato	Increase in the activity of hydrolytic and defence enzymes, improvement of plant nutrition value (polyunsaturated fatty acids contents)
Protection against biotic factors	*Scytonema hofmanni*	rice	Alleviation of salt stress
	Ascophyllum nodosum (extracts)	wheat	Alleviation of drought stress Increase in growth, yield, nutrients uptake
	Sargassum muticum, Jania rubens	chickpea	Alleviation of salt stress Increase in the activity of enzymes and amino acids associated with plant stress responses Improvement of plant growth
	Dunaliella salina (extracted polysacharides)	tomato	Alleviation of salt stress Activation of metabolic pathways involved in plant tolerance to stress Mitigation of the decrease in length and dry weight of shoots and roots
	Oscillaroria agardhii	wheat	Alleviation of drought stress Increase in the activity of hydrolytic and defence enzymes, grain yield Improvement of plant quality parameters

4. RECOMMENDATIONS FOR FUTURE RESEARCH OF MICROALGAE

To advance the field of microalgae-based biopesticides, future research and applied use should focus on the following key areas:

1. Strain Selection and Productivity Optimization

Future research should prioritize strain selection that balances potent in vitro antagonistic activity with high biomass productivity rates. While the order *Nostocales* (specifically the family *Nostocaceae*) is recognized as a metabolically versatile group for secondary metabolite production, their slower growth compared to green algae remains a challenge. Applied research should focus on identified successful strains, which demonstrates both broad-spectrum activity and higher daily biomass productivity.

2. Advanced Analytical and Computational Tools

The discovery of novel lead molecules should be accelerated using high-throughput pipelines such as MS/MS-based molecular networking and dereplication. These tools allow researchers to efficiently identify unique antimicrobial metabolites while eliminating redundant or known compounds. Methods such as high-performance countercurrent chromatography should be explored further to isolate cyclic lipopeptides (e.g., muscotoxins) in quantities sufficient for extensive *in vivo* testing.

3. Economic Viability and Processing

To make micro-algal biopesticides economically competitive with synthetic agrochemicals, researchers must minimize downstream production costs related to harvesting, drying, and biomass processing. It is recommended to focus on water-soluble compounds that can be extracted from dewatered biomass pellets and applied as simple aqueous sprays, avoiding the use of expensive organic solvents.

4. Transition from Lab to Field

A critical area for future research is the transition from controlled laboratory conditions to realistic field applications. Since the production of secondary metabolites in microalgae is tightly regulated by environmental signals, metabolites that are effective in the lab may not be expressed under field conditions. Studies must also account for how environmental stressors—such as pH, moisture, and temperature—impact the stability and persistence of these natural compounds.

5. Safety and Mechanism of Action

Detailed molecular studies are required to understand the mechanisms by which microalgal metabolites, such as exopolysaccharides, activate plant defense signaling pathways (e.g., the salicylic acid and jasmonic acid pathways). Future applications must include rigorous phytotoxicity screenings to ensure that biopesticide candidates effectively control pathogens without eliciting negative effects on crop growth or human and animal health. Finally, research should explore synergistic strain combinations to target specific pathogen communities more effectively than standalone treatments.

4.1 Biostimulants

For the successful implementation of microalgae- and cyanobacteria-based biostimulants (MCBs) in sustainable agriculture, the following recommendations for research and application are suggested:

1. Advanced Molecular and Physiological Research

Future research must prioritize unravelling the biomolecular mechanisms of MCBs using multi-omics approaches (genomics, transcriptomics, proteomics, and metabolomics) to understand how these products induce changes in plant metabolic pathways.

There is an urgent need to define the exact chemical composition of extracts, including the identification of specific bioactive molecules like phytohormones, polyamines, and exopolysaccharides, to reduce product variability and ensure consistent performance. Research should also focus on Implementation high-throughput plant phenotyping to rapidly isolate novel strains with superior biostimulant potential and to characterize their mode of action at different development stages.

2. Optimization of Formulations and Synergy

Research should explore the additive or synergistic effects of combining different microalgal strains, or co-applying microalgae with macroalgae (seaweed) extracts, humic substances, and other microbial inoculants.

Further studies are needed to compare the efficacy of purified bioactive compounds versus crude total extracts, as the latter may benefit from synergistic interactions between diverse metabolites.

3. Field Validation and Applied Use

It is critical to transition from laboratory and greenhouse studies to replicated field trials and whole-field studies to validate efficacy under diverse pedoclimatic conditions, soil types, and environmental stresses. Precise protocols must be established regarding the optimal timing (seed priming vs. defense vs. recovery), dosage, and frequency of application for economically important crops like maize, rice, and wheat.

Studies should investigate how different plant species and even specific cultivars respond to the same MCB to develop tailored agricultural strategies.

4. Sustainability and Circular Economy Integration

To overcome high production costs, MCB production should be integrated into a biorefinery framework, utilizing all biomass components for food, feed, and fuel while using the residual biomass or byproducts (like cell-free supernatants) for agriculture.

Utilize low-cost resources such as industrial or municipal wastewater, anaerobic digestate, and industrial CO_2 for microalgae cultivation to enhance the economic and environmental sustainability of the process.

Rigorous assessments of heavy metals, pathogens, and contaminants are essential, especially for wastewater-grown microalgae, to ensure product safety and compliance with international regulations.

5. Socio-Economic and Regulatory Directions

Use Techno-Economic Assessment and Life Cycle Analysis tools to identify optimal value chains and demonstrate the economic profitability of MCBs to industry stakeholders.

Efforts should be made to streamline international regulations and intellectual property frameworks while raising farm awareness regarding the specific benefits and proper use of bio-based solutions.

4.2 Biofertilisers

Future research and applied use of microalgae-based biofertilisers (MBFs) should focus on addressing existing knowledge gaps, optimizing production costs, and ensuring consistent field performance. The following recommendations are derived from the sources:

1. Advanced Molecular and Physiological Research

Research must transition from observing growth results to understanding the biomolecular pathways through which microalgae influence plants using multi-omics approaches (genomics, transcriptomics, and metabolomics).

Future studies should investigate the mechanisms of "luxury uptake" of phosphorus and iron in microalgae to better exploit their potential as slow-release nutrient carriers.

There is a need to establish a centralized database containing the exact chemical compositions and bioactivity results of various strains to help producers select the most effective candidates for specific crops.

2. Optimization of Production and Sustainability

To reduce production costs, microalgae cultivation should be integrated with wastewater treatment (recovering N and P) and industrial mitigation.

Applied use should adopt a biorefinery model, where high-value compounds (pigments, proteins) are extracted first, and the residual biomass or byproduct (supernatant) is utilized as biofertiliser/biostimulant to increase economic viability. Further research is needed on the use of anaerobic digestate as a low-cost nutrient source for microalgae, creating a closed-loop system for agricultural waste.

3. Field Validation and Application Protocols with Safety and Regulatory Compliance

Laboratory and greenhouse results must be validated through large-scale, replicated field trials across different soil types and climatic conditions to prove reliability to farmers. Applied use, particularly of wastewater-grown biomass, requires rigorous toxicology testing for heavy metals, pathogens (e.g., *Salmonella*, *E. coli*), and cyanotoxins to ensure human and environmental safety.

5. CONCLUSION

The global agricultural sector faces an increasing challenge: the need to produce more food for a growing population while reducing reliance on synthetic chemical pesticides. Synthetic pesticides have led to significant environmental degradation, including soil salinization, water eutrophication, and the emergence of resistant pathogen strains.

The integration of biostimulants and biofertilisers into modern agricultural practices represents a critical shift toward sustainability and the reduction of chemical inputs. While biofertilisers focus on restoring soil fertility and increasing nutrient availability through microorganisms, biostimulants act directly on plant physiology to improve efficiency of nutrients, crop quality, and enhancing adaptability to environmental stressors.

In conclusion, future research must focus on the optimization of strains that balance high biomass productivity with robust bioactivity, while utilizing advanced computational tools like molecular networking to accelerate the discovery of novel compounds. By harnessing synergistic strain combinations and refining application methods such as soil inoculation and foliar sprays, microalgae can be developed into a powerful, environmentally-friendly tool for global disease management

6. PATENTS OVERVIEW

The patent landscape regarding microalgae and cyanobacteria in agriculture has evolved from an initial focus on biofuels to more specific applications in plant growth promotion, plant protection (disease control), weed management, and post-harvest quality improvement. While the commercial implementation of microalgae-based biostimulants has historically faced bottlenecks such as limited research and high production costs, the number of agriculture-related patent applications has seen a significant increase since approximately 2015. Key patents and registered models mentioned in the sources include:

1. Specific Agricultural Formulations and Inoculants

· Utility Model No. 29940 (Czech Industrial Property Office): This model protects a plant growth-promoting product consisting of a specific mixture of bacterial and algal cultures. The mixture includes Bacillus licheniformis, Bacillus megatherium, Azotobacter sp., Azospirillum, Herbaspirillum sp., and the microalga Chlorella vulgaris.

· Horticultural and Agricultural Fertilizers (London Patent No. 663,989): An early historical patent (1952) by Milton, R.F., relating to improvements in fertilizers.

· Compounds of Heavy Metals with Organic Residues (British Patent No. 902,563): Another early patent (1962) by Milton, R.F., focusing on the production of specific organic-metal compounds for agricultural use.

2. Biological Control and Nucleic Acids

· WO2014020624: This patent covers a composition comprising nucleic acids from parasitic, pathogenic, or weed biological systems. It focuses on inhibiting or controlling the growth of these systems using the nucleic acids, a concept that can be applied by using microalgae as carriers for pathogenic fungal DNA to induce disease resistance in crops.

3. Processing and Specialized Applications

· Marine Algae Comminuting (U.S. Patent 4,023,734): This patent (1977) describes a method and apparatus for the mechanical disruption of marine algae to create resulting products.

· Algal Polysaccharides for Medical Use (U.S. Patent 9,119,870): While not agricultural, this 2015 patent for viscosupplementation using algal polysaccharides in the treatment of arthritis demonstrates the high-value potential of microalgal extracts often prioritized by the industry over low-cost agricultural inputs.

The current patent market for microalgal biostimulants is described as being in its infancy compared to macroalgae (seaweeds). However, the push for a circular bioeconomy is driving innovation in utilizing wastewater-grown biomass and biorefinery approaches, which are expected to lead to a new wave of patent filings focused on standardized, well-characterized bio-based products. Researchers emphasize that establishing more patents and standard operating procedures is essential to building market credibility and ensuring the reproducibility of laboratory results in large-scale field applications

Research is moving toward high-throughput discovery pipelines to identify unique metabolites while eliminating redundant known compounds through computational tools like MS/MS-based molecular networking. Despite the technical success of strains like Desmodesmus subspicatus or Tolypothrix tenuis in the lab, consistent field performance and high production costs remain the primary obstacles to full-scale patent commercialization as biopesticides

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