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REVIEW ARTICLE

Harnessing biostimulants from biogas digestates for high-value resource recovery: a review

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Abstract
Improper disposal of organic waste leads to greenhouse gases, pollution, and health risks. Anaerobic digestion offers a sustainable solution by converting this waste into biogas and digestates, which contain valuable nutrients and stimulatory organic compounds that can be recycled to improve plant growth and support food production. Here we review the transformation of liquid and solid digestates into biostimulants by microalgal cultivation, vermicomposting, and insect-based bioconversion. These processes yield phytohormones, polysaccharides, betaines, humic substances, chitin, protein hydrolysates, and growth-promoting microbes, that enhance plant growth and resilience against environmental stresses. Due to the variability in digestate composition, we emphasize the need for optimized formulations, a deep understanding of synergistic interactions among bioactive compounds, and standardized extraction techniques to support broader applications.

Keywords Bioconversion · Bioeconomy · Phytohormones · Microalgae · Sustainability · Vermicomposting · Waste management · Phytohormones

Introduction

One of the major challenges the global community faces today is managing the rising organic waste generated by population growth, intensive agriculture, urbanization, and industrialization [1, 2]. Global waste production is expected to increase from 2.0 billion tons today to 3.4 billion tons by 2050, with 75% of biodegradable waste currently ending up in landfills (37%) or open dumps (33%) [3, 4]. These practices release methane (CH_4) and nitrous oxide (N_2O), contributing significantly to global emissions, while poor waste management leads to pollution, ecological imbalance, health risks, and resource depletion [5–8]. To address these challenges, anaerobic digestion is widely acknowledged as a highly efficient biological process that converts biomass feedstocks into biogas concurrently generating valuable digestate as a residual by-product [9]. While digestate is nutrient-rich and traditionally used as fertilizer, its large volume, variable quality, and logistical complexities related to storage and transportation limit its overall application efficiency in agricultural practices [10]. In this context, an innovative strategy gaining global interest is the production of biostimulants from digestates [11].

Biostimulants are natural substances and microorganisms that, when applied to plants or soil in modest doses, enhance nutrient uptake, efficiency, stress tolerance, and crop quality, independent of their nutrient content [12–15]. Bioconversion of organic substrates has recently proven effective for enhancing biostimulants such as betaines, humic substances, chitin, and chitosan derivatives [16, 17]. Vermicomposting generates humic substances, beneficial microorganisms, and hormone-active compounds, improving soil health and stress tolerance [18, 19]. Similarly, black soldier fly larvae bioconversion, an emerging method for treating solid digestate, increases betaine concentrations in frass and provides bioactive compounds like chitin [17, 20, 21]. Additionally, cultivating microalgae such as *Chlorella* sp., *Scenedesmus* sp., *Dunaliella* sp., *Nannochloropsis sp.*, and *Haematococcus* sp. in liquid digestates supports nutrient recovery and

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biostimulant production [22]. Figure 1 illustrates biostimulant production from digestate via vermicomposting, black soldier fly larvae bioconversion, and microalgae cultivation, highlighting their roles in promoting sustainable waste management and agricultural practices.

This review evaluates current and emerging approaches for recycling anaerobic digestate into diverse biostimulant products within a circular bioeconomy framework. It explores key biostimulant categories and mechanisms derived from digestates, focusing on resource recovery, environmental impact reduction, and sustainable agriculture. The review also highlights the challenges, opportunities, and essential directions for advancing these technologies to enhance the sustainability of agricultural practices.

Anaerobic digestates as a resource for biostimulants

Digestates, the by-product of anaerobic digestion, are semi-stabilized mixtures of solid and liquid fractions comprising partially degraded organic matter, minerals, microbial

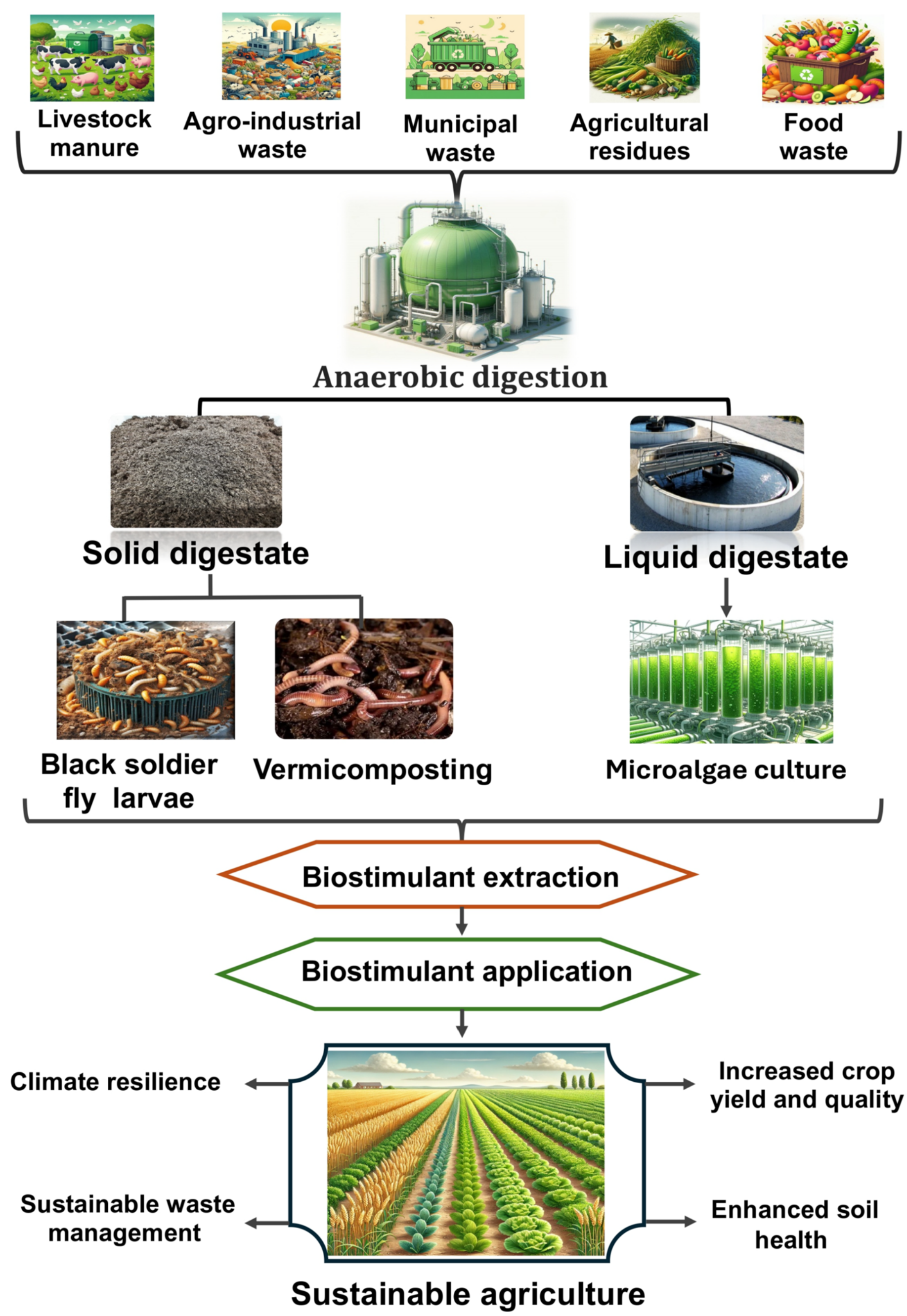

Fig. 1 Upcycling of digestate from anaerobic digestion into biostimulant production for sustainable agriculture. Organic waste streams, including livestock manure, agro-industrial, municipal, agricultural residues, and food waste, undergo anaerobic digestion to produce solid and liquid digestates. These digestates are further processed via bioconversion techniques such as vermicomposting, black soldier fly larvae bioconversion, and microalgae cultivation to generate biostimulant-rich products. The subsequent extraction of biostimulants enables their application in agricultural systems, where they enhance soil structure, microbial activity, and plant growth. This integrated waste valorization approach not only improves crop yield and quality but also contributes to sustainable resource management and climate resilience. By closing the nutrient loop, this system offers a scalable solution for mitigating the environmental impact of waste while supporting agriculture in a more sustainable way

biomass, and numerous bioactive compounds such as amino acids, enzymes, vitamins, and plant hormones [9]. Du et al. [23] reported biostimulants in dissolved organic matter recovered from anaerobic digestion sludge through alkali-hydrothermal treatment with bioactivity for rice growth, attributed to the abundance of amino compounds and humic substances. Another study found auxin-like activities in hydrophobic dissolved organic matter fractions extracted from digestates, comparable to recognized biostimulants [24]. Antón-Herrero et al. [25] treated urban organic waste digestates with strong acid and base solutions to produce biostimulant liquid extracts that improved hydroponically cultivated tomato plant root systems, root hair density, plant weight, and chlorophyll content. These findings highlight the potential of utilizing treated digestates in agriculture to produce fertilizers and biostimulants.

Extraction is a crucial process for isolating and concentrating these specific biostimulant compounds, optimizing their effectiveness in agricultural and environmental implications [26, 27]. Extracting biostimulants allows for customization to meet the particular needs of different crops and growth stages, enhancing their efficacy. Moreover, the extraction process allows to create specific formulations with bioactive compounds, which can reduce the need for synthetic fertilizers and potentially replace mineral fertilizers. The extraction process also addresses logistical challenges, as concentrated extracts reduce weight and volume, making them more practical for transportation, handling, and widespread application in agriculture. Advancements in biotechnology have paved the way for the bioconversion of solid digestates and the microalgal biorefinery of liquid digestates, providing promising approaches for extracting biostimulants from digestates (Fig. 2).

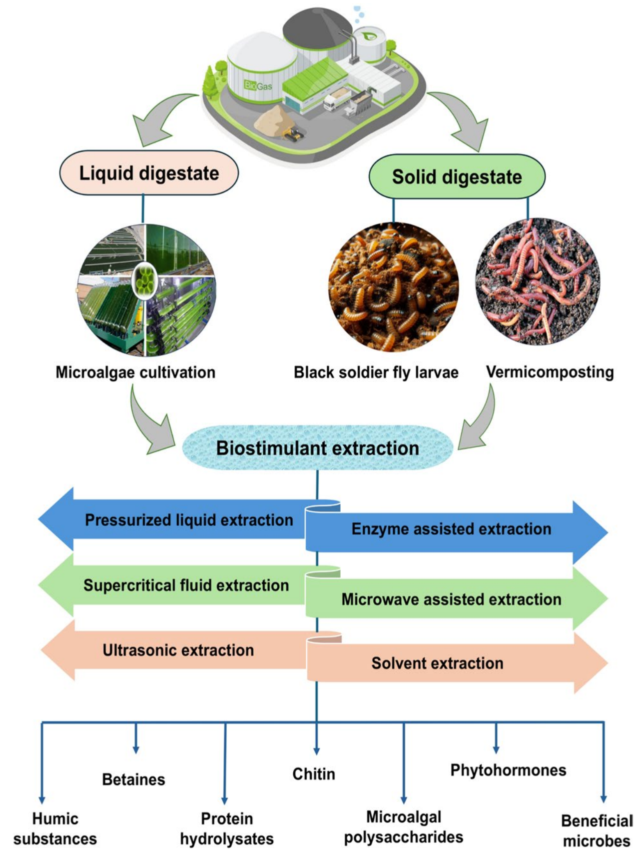

Fig. 2 Key approaches for converting anaerobic digestates into biostimulants products. Liquid and solid digestates, derived from anaerobic digestion, undergo bioconversion via microalgae cultivation, black soldier fly larvae treatment, and vermicomposting, generating biologically active substrates. These substrates are processed using cutting-edge extraction methods such as pressurized liquid extraction, supercritical fluid extraction, enzyme-assisted extraction, microwave-assisted extraction, ultrasonic extraction, and solvent extraction. These methods yield a variety of functional biostimulants, including humic substances, betaines, protein hydrolysates, chitin, microalgal polysaccharides, phytohormones, and beneficial microbes. This extraction process not only valorizes organic waste but also produces bioactive compounds that enhance crop productivity and soil health, contributing to sustainable agriculture

Biostimulant production by microalgal cultures in liquid digestates

Cultivating microalgae on anaerobic digestates presents a highly promising approach for the concurrent treatment of anaerobic digestate while facilitating the recycling of plant nutrients into more valuable algal biomass. This biomass can be processed to extract oils, proteins, feeds, biofertilizers, and bioactive compounds [28, 29]. To date, the cultivation of microalgae on anaerobic digestates has been extensively studied in a wide range of environmental settings and with various types of digestates derived from sludge, manures, and food waste [29, 30]. Notably, digestates contained mineralized nitrogen, predominantly ammoniacal-N, thereby constituting a nutrient source for microalgae [31]. However, the nutrient composition of digestates can vary widely, posing challenges to maintaining optimal nitrogen: phosphorous ratios. Furthermore, the pH of the liquid digestate should be carefully monitored and adjusted as needed to ensure it falls within the optimal range for the chosen microalgae strain. Most microalgae thrive in slightly alkaline conditions (pH 7–9), but specific strains may have different preferences [30].

Both closed and open systems have been used to cultivate microalgae in liquid digestates successfully [32–34]. Closed systems offer precise control over factors such as light, temperature, and nutrient supply, enhancing algal growth conditions. However, photobioreactors in closed systems involve high setup and maintenance costs, increasing initial investment and operational expenses. In contrast, open systems are more cost-effective, scalable, and suitable for larger cultivation areas, making them favourable for commercial-scale microalgal production. However, they are prone to contamination and fluctuations in environmental conditions, which can reduce productivity and consistency. Fernandes et al. [35] demonstrated that kitchen waste-derived digestate could support *Chlorella vulgaris* growth, achieving a maximum growth rate of 0.62% day^{-1} and dry weight increase of 0.86 gL^{-1}. Similarly, Hu et al. [36] reported the feasibility of microalgae cultivation in liquid digestate resulting from anaerobic co-digestion of pig manure and filamentous algae, achieving a biomass productivity of 44.1 mg L^{-1} day^{-1}. However, challenges remain, including the high costs of dilution with water and the operational complexity of microalgal harvesting.

Building on recent advancements, Al-Mallahi et al. [37] developed an innovative microfiltration membrane open reactor system with circular and rectangular configurations enabling efficient nutrients transfer from the digestate reservoir to the cultivation chamber while maintaining 99.9% light transmittance. This system enabled microalgae cultivation using undiluted liquid digestate achieving a growth productivity rate of approximately 45 mg L^{-1} day^{-1}. Similarly, Xu et al. [38] introduced a cost-effective method for microalgal biomass production using municipal anaerobic digestate combined with biomimetic auto-flocculation harvesting system, significantly increasing biomass yield and valuable by-product production. Another study demonstrated that quill board paper serves as a highly effective support material for biofilm cultivation of *Chlorella* sp., resulting in significantly increased biomass yield and productivity, particularly when utilizing anaerobic digestate food effluent as a nutrient source [39]. Furthermore, the addition of natural zeolite to anaerobic digestate was demonstrated as a promising and efficient detoxification strategy, aimed at enhancing microalgal biomass production [40]. Thus, optimizing nutrient utilization, adopting efficient harvesting techniques, and integrating cultivation, harvesting, extraction, and downstream processing can make this approach more economically viable.

Microalgae can accumulate phytohormones intracellularly and exude them into the extracellular milieu [41]. Researchers have correlated the bio-stimulating attributes of microalgal extracts with the presence of primary metabolites such as carbohydrates, proteins, and lipids, essential amino acids like arginine and tryptophan, vitamins, osmolytes like proline and glycine betaine, and polysaccharides [30]. Notably, numerous microalgae strains belonging to diverse taxonomic families, including *Charophyceae, Chlorophyceae, Trebouxiophyceae*, and *Ulvophyceae*, have been characterized by their phytohormone-like activities, encompassing auxins, cytokinins, gibberellins, abscisic acid, and brassinosteroids [42]. Therefore, identifying and selecting microalgal extracts rich in natural phytohormones, particularly auxins and cytokinins, constitute a burgeoning avenue for developing microalgal-based biostimulants [43]. Table 1 summarizes the recent development in microalgae cultured in liquid digestates, key algal species with their biomass productivity, biostimulant components and bio-stimulatory actions in plants.

Extraction of microalgal biostimulants

Extraction methods for microalgal biostimulants are diverse, each with advantages and limitations. It is noteworthy that the optimal choice of extraction method depends on factors such as the microalgal strain, cultivation methodology, timing of microalgae harvest, and intended extraction objectives [26, 44]. A fundamental extraction principle is the preservation of bioactive compounds, with the extraction process being both cost-effective and environmentally benign [45–47]. These techniques aim to obtain high-quality biostimulants rich in beneficial substances such as phytohormones, amino acids, proteins, and other bioactive molecules. The extraction process focuses on maximizing the yield of these compounds, ensuring their bioavailability and efficacy for promoting plant growth, enhancing stress tolerance, and contributing to sustainable agricultural practices.

The initial step in most extraction procedures involves the removal of the microalgal cell wall to release the bioactive compounds [48]. Various mechanical or physical methods can be employed, such as autoclaving, homogenization, microwaving, pulsed electric field technology, sonication, and the use of liquid nitrogen. Additionally, chemical methods, including the use of sodium hydroxide, hydrochloric or sulfuric acids, osmotic shock, nitrous acid, and enzymatic methods using cellulase and protease, are potential protocols for cell wall removal. Enzymatic pre-treatment methods are gaining popularity due to their advantages, despite the higher cost associated with cell lysing enzymes. Nevertheless, mechanical and physical pre-treatment methods are often favoured over chemical or thermal approaches, with bead milling recognized as an effective method for cell disruption [22], and the release of biostimulant compounds [49].

In the subsequent stage, the extraction process aims to isolate the target bioactive compounds using physiologically active chemicals [45, 46, 50]. Traditional solvent extraction methods involve the use of the Soxhlet apparatus, solid–liquid extraction, and liquid–liquid extraction, with hydrophobic solvents such as petroleum ether, aromatic compounds, hexane, cyclohexane, chloroform, acetone, and alcohols (e.g., ethanol, methanol) being the most frequently employed [50]. Among these, the Soxhlet apparatus is widely used for its simplicity, safety, and scalability [51]. However, traditional solvent extraction methods are time-consuming and solvent-intensive and typically yield low product quantities.

In recent studies, organic solvents like acetone and ethanol have shown promise in providing optimal conditions for

Table 1 Biostimulants produced by microalgae culture with liquid digestate with putative plant biostimulation functionality

Digestate origin	Liquid digestate pre-treatment	Microalgal biomass (g dry weight L^{-1} day^{-1})	Cultivated algae species	Biostimulant-containing components	Test plants	Bio-stimulatory actions on crops	References
Swine manure and sewage sludge	Undiluted, Centrifugation, Autoclaving	0.61–4.81	*Chlorella sp.*	Extracted polysaccharides, Aqueous hydrolysate (Proteins, phytohormones)	Wheat	Boosted root length, leaf area, shoot length, photosynthetic pigments, protein and carbohydrate content, fresh and dry biomass	[56, 57]
Food waste	Undiluted, Ozonation, Autoclaving	0.456–4.3			Melon	Altered stem elongation, raised fresh and dry matter in seedling aerial parts	[58, 59]
Poultry litter	Centrifugation, Dilution	0.076–0.61	*Chlorella sp.*	Lipid extraction residual (endogenous phytohormones)	Rice and tomato	Triggered earlier seed germination	[60, 61]
			Scenedesmus sp.	Solvent extract (Unspecified phytostimulants)	Cucumber	Enhanced germination and root growth	[52]
Livestock waste	Ultraviolet light, Filtration	0.26	*Scenedesmus obliquus, Neochloris oleoabundans*	Biomass extracts (Cytokinins, auxins, gibberellins, proteins, and amino acids)	Watercress Bean Cucumber	Stimulated germination, increased growth of stem, roots, and leaf	[62, 63]
Pig manure	Filtration, Dilution	1.039	*Desmodesmus sp.*	Biomass suspension (Phytohormones, cytokinins like zeatin)	Tomato	Increased hypocotyl lengths and volumes	[64, 65]
Livestock waste	Filtration, Autoclaving, Dilution	0.118 1.5	*Scenedesmus sp.*	Microalgal extracts (Phenolic compounds and salicylic acid)	Watercress	Boosted germination index by 10%	[66, 67]
Swine manure	Autoclaving, Mixed with municipal wastewater	0.67		Microalgal hydrolysate (Phytohormones)	Petunia	Enhanced plant growth, increased root, leaf, shoot development, and earlier flowering	[68, 69]
Livestock waste	Filtration, Autoclaving, Dilution	0.311	*Scenedesmus. obliquus*	Microalgal supernatant (Phytohormonal components)	Tomato and barley	Stimulated germination and post germination growth	[70, 71]
Kitchen waste	Dilution, Centrifugation	0.53	*Chlorella SDEC-18*	Microalgal extract (Cytokinin, Auxin)	Lettuce	Improved lettuce seedling growth: increased shoot weight, chlorophyll, carotenoids, proteins, and ashes via altered nitrogen metabolism	[72, 73]
Diluted food waste and animal manure	Dilution	0.042	*Scenedesmus dimorphus*	Microalgal cellular extracts (Phytohormones)	Tomato	Induced germination; impacted antimicrobial activity against bacteria and fungi	[74, 75]

Table 1 (continued)

Digestate origin	Liquid digestate pre-treatment	Microalgal biomass (g dry weight L^{-1} day^{-1})	Cultivated algae species	Biostimulant-containing components	Test plants	Bio-stimulatory actions on crops	References
Piggery effluent	Sand filtered but undiluted	0.017–0.02	*Chlorella sp*	Microalgal cellular extracts (Protein, Carbohydrate, Carotenoids)	Tomato	Elevated mineral content (P, K, Ca, Mg) in fruits; promoted growth	[76, 77]
Organic waste	Filtration, Dilution	0.239	*Dictyospaerium sp*	Microalgal extract (Phytohormones, Amino acids)	Chinese cabbage	Enhanced growth and nutrient uptake	[78]
Winery waste	Centrifugation	0.09	*Chlorella vulgaris*	Polysaccharides, Phytohormones	Wheat	Enhanced germination and root growth	[79, 80]

Digestates undergo pre-treatments such as centrifugation, autoclaving, and filtration to facilitate microalgal cultivation, producing biostimulant-rich extracts. These extracts contain bioactive compounds like polysaccharides, phytohormones, and proteins, which effectively enhance plant growth and development. The biostimulants significantly improve key growth parameters, including germination, root and shoot development, and biomass accumulation. This process demonstrates the potential of digestate-derived biostimulants to support sustainable agriculture

extraction [52]. Modern extraction techniques, such as pressurized liquid extraction, enzyme-assisted extraction, microwave-assisted extraction, and supercritical fluid extraction, have been developed to address the limitations of traditional methods and offer several advantages [45, 46, 53]. Supercritical fluid extraction is an alternative with low environmental impact in comparison with conventional extraction methods, which often involve the use of substantial amounts of hazardous chemicals, including chlorinated solvents [53, 54]. One of the most significant advantages of supercritical fluid extraction lies in its markedly reduced, and often eliminated, reliance on toxic organic solvents. The prevalent choice for the solvent in supercritical fluid extraction is carbon dioxide (CO_2), which further contributes to the environmentally friendly profile of the technique compared to other extraction techniques [55]. Overall, a diverse range of extraction methods, from traditional solvent-based techniques to advanced approaches like supercritical fluid extraction, provides effective and sustainable options for isolating high-quality biostimulants from microalgae.

Biostimulant production by composting of solid digestates

Vermicomposting of solid digestates

The vermicomposting process involves the bio-oxidation and stabilization of organic materials, facilitated by the symbiotic interactions of bacteria, earthworms, and other fauna [19]. Notably, earthworms play a pivotal role in reshaping the microbial community, diversifying its activities and populations, and producing nutrient-rich mucus as a vital microbial food source. Moreover, earthworms contribute to the biochemical degradation of organic matter, promoting enhanced aeration conditions, substrate fragmentation, and a substantial augmentation of microbial activity [81]. Through the digestive process, earthworms facilitate the mineralization of calcium and nitrogen, which subsequently react with carboxylic or phenolic groups within the produced acids, forming humic acid. The presence of iP-type cytokinins in earthworms' faeces (vermicomposts) can be attributed to the gut microbiota within the digestive system, which serves as precursors to Z-type cytokinins [19, 26, 82, 83]. These cytokinins function as advantageous phytohormonal signals, coordinating essential cell cycle checkpoints that eventually increase cell proliferation and plant growth [19, 84].

Vermicompost leachates, extracts, or teas are liquid solutions rich in water-soluble compounds, nutrients, and microbial populations, obtained through the extraction process [85–87]. Leachate is formed by excess moisture in vermicompost, and extracts are obtained by soaking vermicompost in water, offering the potential for biostimulant extraction. Moreover, the tea, generated by aerating vermicompost and water, enhances microbial biomass, with resulting products potentially serving as a potent microbial additive. For instance, García et al. [88] demonstrated that vermicompost-based biostimulants contain high concentrations of humic substances especially humic acids and fulvic acids, which enhanced soil nutrient availability and water retention, thereby promoting plant growth. Similarly, Koskey et al. [89] demonstrated that the application of vermicompost extract has a bio-stimulatory effect on soil mycorrhizal activity, resulting in a significant enhancement in crop productivity. The bio-stimulatory activity of vermicompost is attributed to a synergistic effect of many plant hormones, including cytokinins, auxins, abscisic acid, gibberellins, brassinosteroids, and other unidentified beneficial substances [19, 90].

Recently, vermicompost leachate has garnered attention as an effectively and economical biostimulant [26, 82]. It encompasses advantageous microbial populations and humic substances, particularly well-suited to stimulate plant growth [91, 92]. Dube and co-workers [93] demonstrated that vermicompost leachate positively affects the growth, nutritive composition, phytochemical content, and antioxidant potential of cultivated *Drimiopsis maculata*, through the bio-stimulatory effect. Numerous investigations have substantiated the presence of phytohormones, such as auxins, cytokinins, gibberellins, and brassinosteroids, alongside phenolic acids including protocatechuic acid, p-hydroxybenzoic acid, p-coumaric acid, and ferulic acid, within vermicompost lechate [94, 95]. Furthermore, vermicompost leachate has demonstrated its efficacy in reducing sodium ion (Na^+) accumulation in salt-stressed plants, elevating phytonutrient levels and antioxidant activity, improving soil characteristics, and mitigating nutrient deficiencies across a range of crop species [18, 96]. Overall, these findings underscore the diverse and valuable contributions of vermicomposting of solid digestate as a useful source for extracting plant biostimulants, demonstrating its potential to play a pivotal role in enhancing agricultural productivity.

Insect-based bioconversion of solid digestates

Insect-based bioconversion has emerged as a promising method for recycling organic waste, including solid digestates, into valuable biostimulants and animal feed components [1, 97, 98]. Black soldier fly larvae are among the most commonly used insects for bioconversion due to their high tolerance for various waste types, including solid digestates [21, 99, 100]. Black soldier fly can colonize diverse habitats, encompassing manure and crop residues while yielding valuable products for agricultural practices, such as fertilization and feed for livestock and aquaculture [101, 102]. For instance, Spranghers et al. [103] investigated the feasibility of rearing black soldier fly larvae on various substrates, including solid digestate. While black soldier fly larvae were able to develop on digestate, their total biomass was comparatively lower than when reared on alternative substrates, such as food waste. Solid digestate typically contains lignocellulosic material, which is challenging for the larvae to convert into simple sugars, hindering their growth.

To enhance the efficacy of black soldier fly larvae in digestate valorization, pre-treatment of solid digestates is recommended to break down lignocellulosic fibres, making nutrients more accessible to the larvae. For instance, although larvae fed on hydrolysed digestate showed lower biomass compared to a standard diet, they nearly doubled in size compared to those on untreated digestate [104]. However, the study further reported that enzyme-treated digestate did not result in efficient larval growth when compared to a standard diet, necessitating the development of a more effective pre-treatment method [104]. A study exploring the integration of pre-treated crop residues for optimizing the valorization of two distinct biogas digestates by the black soldier fly larvae observed significantly diminished larval growth on two diets derived from digestates in comparison with a standard diet [105]. However, a substantial difference in larval length gain was identified between the two digestates, highlighting that specific digestates influence black soldier fly larvae yield differently. This underscores the necessity for case-specific composition evaluations, considering the inherited chemical attributes of the digestate.

Furthermore, Elsayed et al. [106] observed that the growth and development of black soldier fly larvae were influenced by varying the ratio of solid digestate to larval biomass. When black soldier fly larvae were cultivated using solid digestate to larval biomass of 0.25, 0.50, 0.75, and 1.00, an increase digestate ratio corresponded to heightened growth and accelerated developmental rates of the larvae. Similarly, Fu et al. [100] conducted a study with food residues digestate that demonstrated superior suitability as a substrate for rearing black soldier fly larvae. The more efficient larval growth was attributed to the elevated crude protein content ($34.5 \pm 2.4\%$) of the digestate, leading to significantly higher individual larval weights. Moreover, Bosch et al. [107] recommended the use of 5-day-old larvae when proposing standardization approaches for black soldier fly larvae feeding studies. Older larvae exhibited heightened capacity to consume challenging, less assimilable feedstocks, resulting in increased food consumption compared to their younger counterparts [108].

The valorization of pre-treated solid digestate through black soldier fly produces two primary products: frass and larval biomass [109]. Notably, worm cast and frass have been identified as sources of multiple biostimulants [21, 110]. Frass contains humic acids, which undergoes biochemical transformations, resulting in the release of diverse biostimulants including auxins and cytokinins [21]. Additionally, Spranghers et al. [103] demonstrated that the protein content of black soldier fly larvae varies within the range of 399 to 431 g kg^{-1} of dry matter and providing valuable source of protein hydrolysate-based biostimulants. Besides, pupal exuviae from insects like black soldier fly have become a valuable biomass source for chitin and chitosan production [111]. Chitin-rich by-products can be obtained from various sources, including larval exoskeleton, pupal exuviae, and deceased pupae [112]. Additionally, the frass produced by black soldier fly larvae is abundant in microorganisms [20], and many of these microorganisms possess bio-stimulatory capabilities [113]. Overall, black soldier fly bioconversion of pre-treated solid digestates yields valuable products such as frass and larval biomass, both rich in biostimulants and nutrients, supporting sustainable agriculture through enhanced plant growth, soil health, and nutrient recycling.

Biostimulant extraction from bio-converted solid digestates

The extraction of biostimulants from bio-converted solid digestates, such as those processed through vermicomposting or insect-based bioconversion, is a crucial step in maximizing the utility of these materials for agricultural applications. Researchers have developed various extraction techniques to solubilize humic substances, including ultrasound, microwave, magnetic stirring, mechanical shakers, or aqueous alkali solutions for humic acids extraction [114]. Notably, the alkaline solvents technique, pioneered by the International Humic Substance Society, stands out as an effective method for extraction [24]. Following the alkaline extraction method, Aguiar et al. [115] utilized a 0.5 mol L^{-1} sodium hydroxide solution under a nitrogen atmosphere, followed by overnight shaking, to extract humic acids. The extracted material was then subjected to centrifugation, acidification with 6 mol L^{-1} hydrochloric acid, purification through rinsing and further centrifugation, followed by neutralization, dialysis, and lyophilization to isolate the humic acids. However, Jana et al. [116] demonstrated that the column-based continuous elution method for extracting humic acids from vermicompost of organic waste using 0.2 M sodium hydroxide as the extractant yielded 74.7% and 97.9% extractable humic acids in 24 h and 48 h, respectively, surpassing the 47.5% and 63.3% yields obtained by the International Humic Substance Society method. Studies utilizing alkali as extractants have shown differing molecular characteristics and composition in humic acids based on the specific extractants employed [117]. Hence, the careful selection of the extractant for humic acids extraction is imperative.

Furthermore, Zlotka et al. [118] developed a chitin extraction method from black soldier fly larvae involving drying, grinding, deproteinization, demineralization, and depigmentation. Deproteinization, essential for chitin isolation, is typically achieved with sodium hydroxide, while demineralization uses hydrochloric acid to remove mineral residues. Depigmentation can involve agents like hydrogen peroxide or potassium permanganate to remove coloration, depending on the application. The product undergoes sodium hydroxide neutralization and water rinsing post-filtration. Purified chitin is desiccated at around 60 °C for moisture removal and integrity preservation. In contrast, Soetemans et al. [119] recommended drying at 105 °C for 48 h for complete moisture removal. However, Lin et al. [120] noted environmental concerns with current chitin extraction via hydrochloric acid and sodium hydroxide. The major drawback of this method lies in the substantial production of considerable quantities of acid and alkaline effluents, necessitating additional treatment before release. Addressing environmental concerns, Lin et al. [120] proposed an eco-friendly microbial fermentation using *Bacillus licheniformis A6* on black soldier fly pupal exoskeletons, yielding a 12.4% chitin recovery rate with glucose as the medium, reducing the need for acid and alkaline effluents.

Various enzymes, including alcalase from microorganisms, catalyse protein hydrolysis, vital in producing protein hydrolysates through chemical or enzymatic processes [121]. Firmansyah and Abduh [122] reached a 10.7% yield employing bromelain for protein hydrolysates synthesis from black soldier fly larvae, while Rini [123] achieved a 31% yield using 6% papain enzyme. In a recent systematic investigation by Abduh et al. [124], the extraction of protein hydrolysates from black soldier fly larvae involved pulverizing the larvae, combining them with cold Aquades, and subsequently using acid and alkaline solvents for extraction. After centrifugation, the pH was adjusted to 7 by adding either a 0.25 M hydrochloric acid or 0.25 M sodium hydroxide solution to the supernatant. Protein hydrolysates were achieved using a phosphate buffer solution (pH 8) containing bromelain enzyme at a 1:10 concentration ratio. These approaches highlighted the potential of black soldier fly larvae as a promising bioactive hydrolysate source. Yet, further research is necessary to optimize protein recovery and hydrolysis conditions for enhanced protein hydrolysates production. Table 2 summarizes biostimulant components derived by extraction of bio-converted solid digestates and their bio-stimulatory actions in plants.

Major biostimulants produced by bioconversion of digestates

Phytohormones

Utilizing microalgal extracts containing phytohormones holds significant promise for developing

Table 2 Biostimulants extracted from digestates and bio-converted solid digestates

Biostimulants origin	Biostimulant component(s)	Test plant	Application rate	Bio-stimulatory effects on crops	References
Vermicompost leachate	Phytohormones	Tomato	18 ml L^{-1} water dilution	Enhanced growth, reduced Na + in salt-stressed plants, improved stomatal conductance, and delayed senescence	[18]
Vermicompost	Humic substances	Garlic	1:40 (v: v) ratio	Improved the productive, commercial, and internal quality parameters	[125]
Vermicompost leachate	Unspecified	Wild onion	1:10 for leaves and 1:20 bulbs	Enhanced plant growth improved the leaves and bulbs	[93]
Vermicompost extracts	Humic acid Fulvic acid	Hemp	Humic acid: 0.05 mg mL^{-1} Fulvic acid: 1%	Stimulated seed germination and hypocotyl and radicle growth, increased chlorophyll concentration	[126]
Vermicompost	Humic substances	Lettuce	15 mg L^{-1}	Boosted lettuce yield, protein, nitrate uptake and enhanced enzyme activity	[127]
Chicken manure-based vermicompost	Mixture	Tomato and lettuce	–	Sped up vegetable seed germination and accelerated growth	[128]
Sludge digestate	Amino acids, Humic substances	Rice	–	Promoted plant growth	[23]
Vermicompost liquid extract	Auxins, cytokinins, and Humic acids	Begonia, sugarcane, and mint	1–2% of vermicompost liquid extract water dilution	Increased rooting stem cuttings	[16]
Vermicompost of black soldier fly larval frass	Unspecified	Pine	1% aqueous solution	Increased resin yield	[129]
Black soldier larval frass	Growth promoting microbes	Lettuce	Frass and biochar at a 10:90 (v: v) ratio	Promoted 1.6–6.8 times more lettuce growth	[130]
Sewage sludge digestate	Humic-like substances Fulvic acid	Lettuce	4.6 mg L^{-1} of dissolved organic carbon	Increased aerial biomass by average ranged from 7 to 30%	[131]
Black soldier fly larvae	Chitin, chitosan	Tomato		Enhanced resistance against bacterial wilt	[132]
Vermicompost	Humic substances	Arabidopsis	1 mg L^{-1} of humic substances	Enhanced protein and energetic metabolism resulting in higher growth	[133]
Vermicompost liquid extract	Beneficial microbiome and biomolecules	Clover, Lentil, Sunflower	25.6 L ha^{-1} (1:40) vermicompost liquid extract: water dilution	Enhanced microbial presence, elevated shoot biomass and increased grain yield (+ 37%)	[89]
Vermifiltration of solid digestate	Mixture	Lettuce	30% dilution of the aqueous extract	Increased germination index	[134]
Vermicompost leachate	Bioactive compounds	Common bean	10% vermicompost leachate	Increased pod production and biocontrol of root rot	[135]
Vermicompost of sludge digestate	Fulvic acid, Indoleacetic acid, Jasmonic acid	Rice	10% vermicompost extract	Promoted root growth	[136]

Solid digestate bioconversion yields bioactive compounds, including phytohormones, humic substances, amino acids, and growth-promoting microbes. These compounds enhance plant growth by stimulating root development, promoting seed germination, and increasing biomass accumulation, while also boosting stress resistance. When applied at various rates and through different methods, these biostimulants exhibit strong bio-stimulatory effects, improving nutrient uptake, metabolic activity, and accelerating plant growth cycles. This showcases their potential to significantly enhance crop performance and sustainability in agricultural systems phytohormone-based biostimulants for use in crop production. These phytohormones can influence a wide array of metabolic functions, encompassing nutrient uptake, photosynthesis, and nucleic acid synthesis [137, 138]. Notable examples of algal cytokinins include zeatin, zeatin riboside, kinetin, iso-pentenyladenosine, and 6-benzylaminopurine [139, 140]. Certain microalgae, such as *Scenedesmus sp*., exhibit high concentrations of zeatin riboside and isopentenyl adenine, while *Arthrospira sp.* contains notable levels of *trans*-zeatin [141]. Microalgal-derived cytokinins are known to greatly facilitate essential physiological processes in plants, including cell division, development, differentiation, organogenesis, dormancy, seed germination, ageing, leaf pigmentation, and responses to biotic and abiotic stimuli [50].

Brassinosteroids, a recently defined class of substances, serve a dual role as growth-promoting hormones and stress mitigators [142]. These hormones play critical roles in various plant activities, including pollen tube elongation, vascular differentiation, leaf bending, and seed germination [143]. Notably, brassinolide and catasterone are two brassinosteroids associated with microalgae. Concentrations of brassinosteroids in microalgae vary, with levels ranging from 117.3 pg g^{-1} dry weight in *Raphidocelis subcapitata* (Mosonmagyaróvár Algal Culture Collection 317) to 977.8 pg g^{-1} dry weight in *Klebsormidium flaccidum* (Mosonmagyaróvár Algal Culture Collection 692) [144]. Moreover, brassinosteroids interact synergistically and additively with auxins and gibberellins, influencing ethylene production and facilitating diverse metabolic and signalling functions in plants [144].

Microalgal polysaccharides

Microalgae can synthesize a diverse array of polysaccharides. Polysaccharides have a multifaceted impact, stimulating root growth, improving nutrient availability, enhancing photosynthesis, increasing stress tolerance, and acting as signalling molecules [145, 146]. The stimulatory effects of microalgal polysaccharides are often mediated by their ability to activate signalling pathways depending on microbial-related molecular patterns [147]. El Arroussi et al. [148] found that exopolysaccharides from *Dunaliella salina* activate defence pathways in tomato plants under salt-induced stress. In a study by Farid et al. [149], *Chlorella vulgaris* polysaccharides applied to tomato plants increased ascorbate peroxidase enzymatic activity, leading to enhanced antioxidant defence mechanisms. This elevation in ascorbate peroxidase activity indicated an improved capacity to scavenge reactive oxygen species, thereby mitigating oxidative stress and promoting overall plant resilience. However, the fundamental challenge in employing microalgae polysaccharides as plant biostimulants in agriculture is our incomplete understanding of the structural and functional effects of these compounds on plants.

Betaines

Betaines are recognized as natural plant biostimulants with potential applications in agriculture, either through direct application or as fertilizer additives [150]. These compounds, initially categorized under seaweed extract by du Jardin [13] are not limited to seaweed but are found across various organisms, including plants, animals, and microorganisms. Notably, taurine betaine and gamma-aminobutyric acid betaine have been identified in earthworms [151]. Betaines play a vital role in stabilizing enzymes, protecting protein structures, and fortifying lipids and membranes [152]. Consequently, betaines have demonstrated significant efficacy in enhancing tolerance to diverse environmental stressors such as salinity, drought, and oxidative stress [17]. In bioconversion compost, betaines, including valine betaine and gamma-aminobutyric acid betaine, were identified, with larvae bioconversion significantly increasing their levels, making betaines a promising component of plant biostimulants [17]. Their multifaceted functions underscore their importance as protective biostimulants, providing increased resilience against adverse environmental conditions and potential applications in stress mitigation strategies.

Humic substances

Humic substances encompass a group of natural soil organic compounds particularly humic acids and fulvic acids originating from the decomposition of plants, animals, and microorganisms, and the metabolic activities of soil microbes. These diverse and complex compounds engage in intricate interactions with soil microbes, which are further influenced by plant roots and their exudates. Vermicompost and other bioconversion compost materials serve as valuable sources of humic substances that find extensive applications in agriculture as humic substances-based biostimulants [126]. Epelde et al. [153] identified humic acids in vermicompost, comprising 4% to 17% of its total weight. Humic substances are primarily recognized for their bio-stimulatory actions, notably the enhancement of macro- and micro-nutrient uptake via increased soil cation exchange capacity, often referred to as humic substance-facilitated root nutrition. These effects arise from complex interactions among humic substances, plant roots, and rhizosphere microbes, which collectively enhance plant growth and yield [154, 155].

Humic substances play a crucial role in regulating plant stress-related hormones, such as auxin, jasmonic acid, and abscisic acid, impacting phytohormone-mediated processes in plant roots [156]. They are reported to regulate reactive oxygen species concentration and superoxide dismutase genes in the cytosol, fostering cell growth and differentiation [88]. These bio-stimulatory actions extend to trigger signalling pathways mediated by auxin and nitric oxide, leading to the up-regulation of various auxin-regulated genes in plant roots [157, 158]. Additionally, with their higher molecular masses, humic substances can enhance key enzyme activities that modulate stress responses [159]. They can reduce hydrogen peroxide, lipid peroxidation, and increase proline content, promoting stress-responsive microbial communities in the rhizosphere, especially in the context of salinity and drought stress [160].

Chitin

Chitin and chitosan are bio-based polymers with versatile applications in agriculture, cosmetology, food, paper, pharmacy, and textiles [112, 161]. Chitin has been observed to have various bio-stimulatory effects on plant growth and stress tolerance, often associated with increased photosynthetic activity, abiotic stress tolerance, antioxidant enzyme activity, and the expression of defensive genes [162]. Recently, the cost-effective production of chitin through larval conversion, particularly from the black soldier fly larvae has garnered widespread interest [111, 163]. In addition, chitin-rich by-products can be derived from the larval exoskeleton, pupal exuviae, and dead pupal accumulation [164]. Previous studies have also demonstrated that chitin constitutes 10–15% of the dry matter of insects [103]. Recently, Hahn et al. [112] extracted chitin with approximately 85% purity from black soldier fly pupal exuviae, yielding 14% relative to the original insect biomass. Chitin and chitosan were effectively derived and analysed from various black soldier fly larvae biomasses, with pupal exuviae noted for their high yields and easy accessibility, making them ideal for this purpose [165]. Furthermore, Rampure et al. [111] recently demonstrated that chitin from commercial shrimp and black soldier fly sources has similar chemical structures and physicochemical properties, both showing α-chitin orientation.

Protein hydrolysates

Protein hydrolysates are mixtures of peptides, amino acids, enzymes, and bioactive compounds [166]. Bioconversion of organic waste by organisms such as earthworms and insect larvae generate abundant protein hydrolysates through their growth and metabolic processes [166]. Firmansyah and Abduh [122] used bromelain as a biocatalyst to produce 50% protein hydrolysates from defatted black soldier fly larvae, achieving high levels of essential amino acids like lysine, leucine, and valine. Protein hydrolysates have been shown to enhance nutrient uptake by stimulating soil microbial and enzymatic activity, increasing micronutrient availability and mobility, modifying root architecture, and boosting enzymatic activities involved in key processes such as nitrate reduction, glutamine synthesis, and Fe (III)-chelate reduction [43, 167, 168]. Furthermore, protein hydrolysates act as signalling molecules that initiate and modulate plant physiological and molecular processes [166, 169, 170]. In summary, protein hydrolysates are potent biostimulants that enhance nutrient uptake, stimulate soil and plant processes, and act as signalling molecules to promote plant growth and resilience.

Plant growth-promoting microbes

The isolation and application of microbial biostimulants from digestate bioconversion hold significant potential for modern agriculture, with advanced sequencing methods like metagenomics enabling precise identification of plant growth-promoting microbes [171]. Following the screening process, selected beneficial microbes, which may include nitrogen-fixing bacteria, phosphate-solubilizing microorganisms, or plant growth-promoting fungi, are isolated and cultured under controlled conditions to ensure purity and viability [172, 173]. For instance, Mang et al. [174] found greater microbial diversity in bioconversion compost from agricultural residue-derived digestate compared to farm-derived compost. These microbial biostimulants confer several advantages when applied to agricultural systems, including enhanced nutrient uptake, improved stress tolerance, increased root development and regulation of phytochromes [175, 176]. Field trials have consistently demonstrated increased crop yields when these microbial biostimulants are introduced, showcasing their potential as sustainable contributors to modern agriculture [177, 178]. Karnwal [179] cultured and screened zinc-solubilizing bacteria isolated from vermicompost, highlighting their ability to enhance zinc bioavailability and, in turn, promote tomato growth. Nonetheless, microbial biostimulants from digestate bioconversion hold a potential that requires quality control and formulation optimization.

Effects of digestate-derived biostimulants applied in agriculture

Plant growth improvement

Sustainable agriculture seeks to balance rising food demand and protecting natural resources. Numerous studies have shown that biostimulants from bioconversion of composts and microalgae effectively enhance crop yield, quality, and improve plant stress tolerance [180, 181]. For instance, *Chlorella vulgaris* and *Spirulina platensis* at a rate of 3 g dry powder per kg soil enhanced dry weight of maize plants by 30% and 35%, respectively [182]. In a recent study, six Nordic microalgae bio-stimulatory impact was assessed using *Arabidopsis thaliana* root growth assays and greenhouse experiments with *Lactuca sativa* L. [183]. The most potent hydrolysed extracts resulted in an 8%–13% increase in *Arabidopsis* root elongation and a 12–15% rise in lettuce yield. Notably, these extracts also demonstrated improved photosynthetic performance in lettuce, with a 26%–34% increase in photosystem II quantum yield and a 34–60% rise in thylakoid proton flux [183]. Similarly, Jimenez et al. [184] reported that applying microalgae resulted in a substantial increase of 32% in tomato plant weight and 13% in chlorophyll a content, as compared to untreated soil conditions. A recent study demonstrated that cultivating *Chlorella vulgaris* in digestate sludge and wastewater concentrate produces a high-value biostimulant, enhancing germination, root formation, and chlorophyll production in plants [185].

Humic substances have exhibited positive effects on the growth of wheat, peas, and other plants, attributed to their ability to stimulate nutrient absorption, induce root structural changes, and modify soil conditions such as pH, cation exchange capacity and water holding capacity [154, 157, 186, 187]. Vermicompost liquid extract, used as foliar treatment, enhanced organic lettuce yields by modulating metabolic processes. This manifested as reduced carbohydrate levels, increased leaf protein content, and elevated activities of nitrate reductase and phenylalanine ammonia-lyase [127]. Balmori et al. [125] demonstrated that foliar application of humic liquid extract from vermicompost enhances garlic (*Allium sativum L.*) quality by augmenting bulb size, garlic clove count, and internal fruit attributes. Alternatively, products originating from black soldier fly larvae, encompassing the larvae, and their frass, recognized for their protein abundance, have been investigated as potential reservoirs of plant biostimulants [20]. Fundamental mechanisms underlying the effect of biostimulants on enhancing crop growth, productivity, and quality are depicted in Fig. 3.

Abiotic stress tolerance

Plants frequently face various abiotic stresses, such as drought, heat, salinity, chilling, freezing, oxidative, and nutrient deficiencies, which can negatively impact their metabolism and crop productivity [188]. Biostimulants are crucial in preparing plants to respond more effectively to these impending stresses [137]. Fundamental mechanisms underlying the effects of biostimulants on enhancing abiotic stress tolerance in plants are depicted in Fig. 4. Phytohormones like auxins, gibberellins, cytokinins, and abscisic acid regulate plant growth, development, and responses to stresses. Biostimulants can modulate the production, transport, and signalling of these hormones [110, 189, 190]. For example, plant processes such as root development and drought tolerance are regulated by an intricate interplay between different phytohormones. Hormonal adjustments assist plants in the strategic allocation of resources and adaptation to stressors [191]. The study conducted by Benazzouk and colleagues [18] demonstrated that applying vermicompost leachate can alleviate the negative effects of salt on leaf senescence. The ameliorative effects were mostly attributed to the modulation of endogenous phytohormones, rather than the passive absorption of exogenous hormonal substances.

Reactive oxygen species, including superoxide radicals and hydrogen peroxide, accumulate in plant cells during periods of stress, potentially causing cellular damage. Biostimulants containing antioxidant-rich compounds have been found to mitigate this damage by scavenging reactive oxygen species and protecting plant cells from oxidative stress. Biostimulants maintain cellular structures by stimulating enzymes such as superoxide dismutase and catalase, which aid in detoxifying reactive oxygen species [192]. For example, vermicompost extract has shown the ability to activate antioxidant enzymes and enhance reactive oxygen species scavenging in rice (*Oryza sativa* L.) [193]. Similarly, using sulphated exopolysaccharides from the halophile green microalgae *Dunaliella salina* significantly improved tomato plants' resilience to salt stress by increasing the activity of antioxidant enzymes, including superoxide dismutase, peroxidase, and catalase [148]. Biostimulants also enhance the activity of photosynthetic enzymes, increase chlorophyll levels, and safeguard chloroplasts against damage induced by stress, resulting in improved photosynthetic efficiency. For example, foliar application of protein hydrolysates has been demonstrated to ameliorate drought stress by regulating stomatal aperture, preserving chloroplast ultrastructure, facilitating osmotic adjustments, and enhancing antioxidant systems [194].

Moreover, biostimulants play a pivotal role in inducing changes in root architecture, thereby improving mechanisms for nutrient uptake and facilitating more efficient nutrient transport within the plant [195]. These adaptations bolster the plant's capacity to maintain essential metabolic processes and uphold a healthy nutrient equilibrium, particularly during periods of stress. Plants possess intricate networks of stress signalling pathways within their cells that enable them to detect and respond to various stressors, as extensively reviewed by Ahmad et al. [196]. Biostimulants can modulate these pathways by activating specific protein kinases, receptors, and second messenger molecules [197]. This modulation triggers defence mechanisms leading to the expression of stress-related genes and an overall enhancement in stress tolerance.

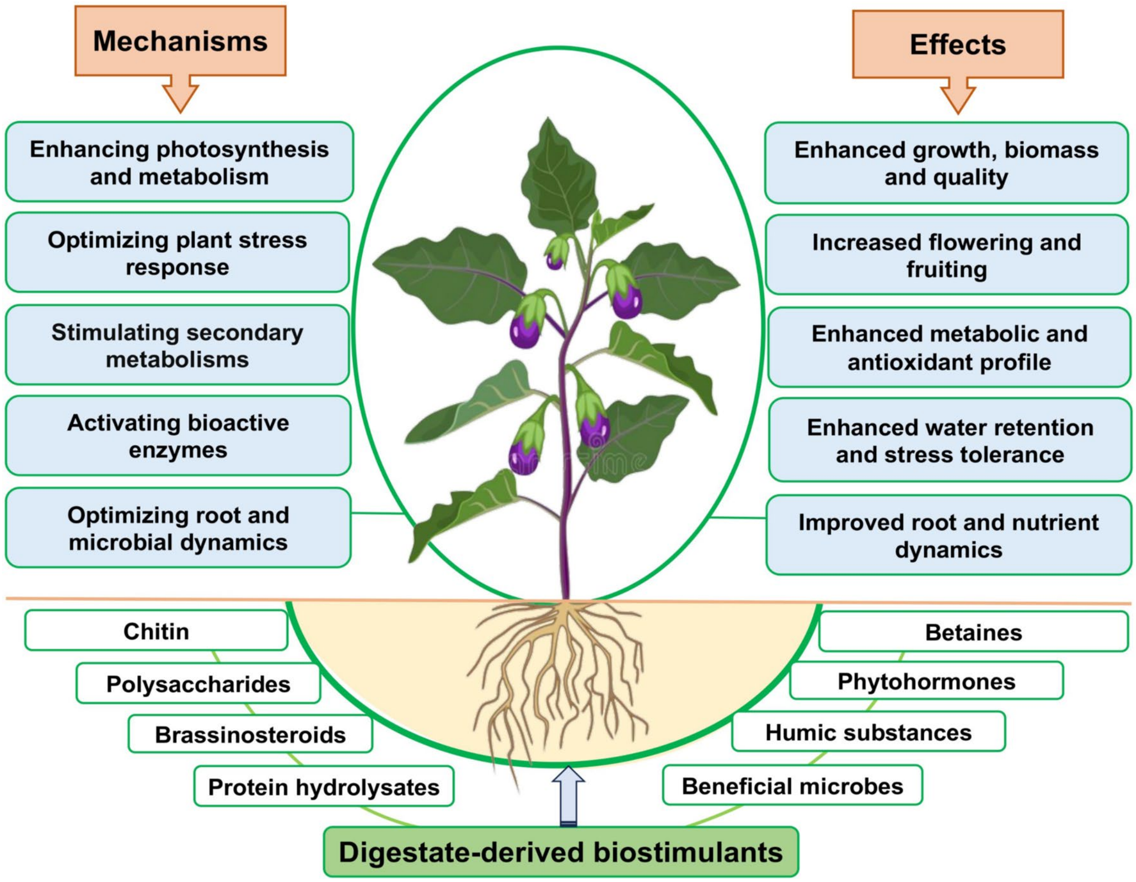

Fig. 3 Mechanisms of digestate-derived biostimulants on crop physiology, growth, and crop quality. Digestate-derived biostimulants, including chitin, polysaccharides, brassinosteroids, protein hydrolysates, betaines, phytohormones, humic substances, and beneficial microbes, exert their effects by enhancing photosynthesis, optimizing plant stress response, stimulating secondary metabolism, and activating bioactive enzymes. These processes lead to optimized root-microbe interactions, improving nutrient uptake and overall plant health. The biostimulants contribute to increased biomass, enhanced growth and fruiting, and improved crop metabolic and antioxidant profiles. Additionally, they boost water retention and stress tolerance, showcasing their potential to enhance both productivity and crop resilience, making them critical components for sustainable agricultural systems

Perspective

Environmental sustainability

The production of biostimulants from anaerobic digestion by-products offers numerous potential benefits across agricultural, environmental, and economic domains. Firstly, it provides a sustainable solution for managing organic waste by valorizing the nutrient-rich residues of anaerobic digestion, thus reducing reliance on landfill disposal, and decreasing environmental pollution. Secondly, biostimulants from these by-products enhance soil fertility and structure, promote nutrient availability, and stimulate beneficial microbial activity, leading to improved crop yields, quality, and resilience to environmental stresses. Additionally, by reducing the need for synthetic fertilizers and pesticides, biostimulant use contributes to preserving soil health and biodiversity, mitigates nutrient runoff and eutrophication of water bodies, and lowers greenhouse gas emissions associated with the production of synthetic fertilizers. For instance, utilizing organic waste through bioconversion methods, such as vermicomposting and larval bioconversion composting, reduced greenhouse gas emissions compared to conventional composting practices [198]. Bioconversion methods involving earthworms or larvae mechanically aerate the soil, enhance carbon sequestration, and reduce methane and nitrous oxide emissions, contributing to decreased greenhouse gas emissions [199].

Furthermore, microalgae exhibit efficient biosorption of pollutants, due to the distinctive chemical makeup of their cell walls, where cellulose, alginate, chitin, and glycan offer essential sorption sites for contaminants. A recent study demonstrated that mixotrophic algal cultivation in liquid

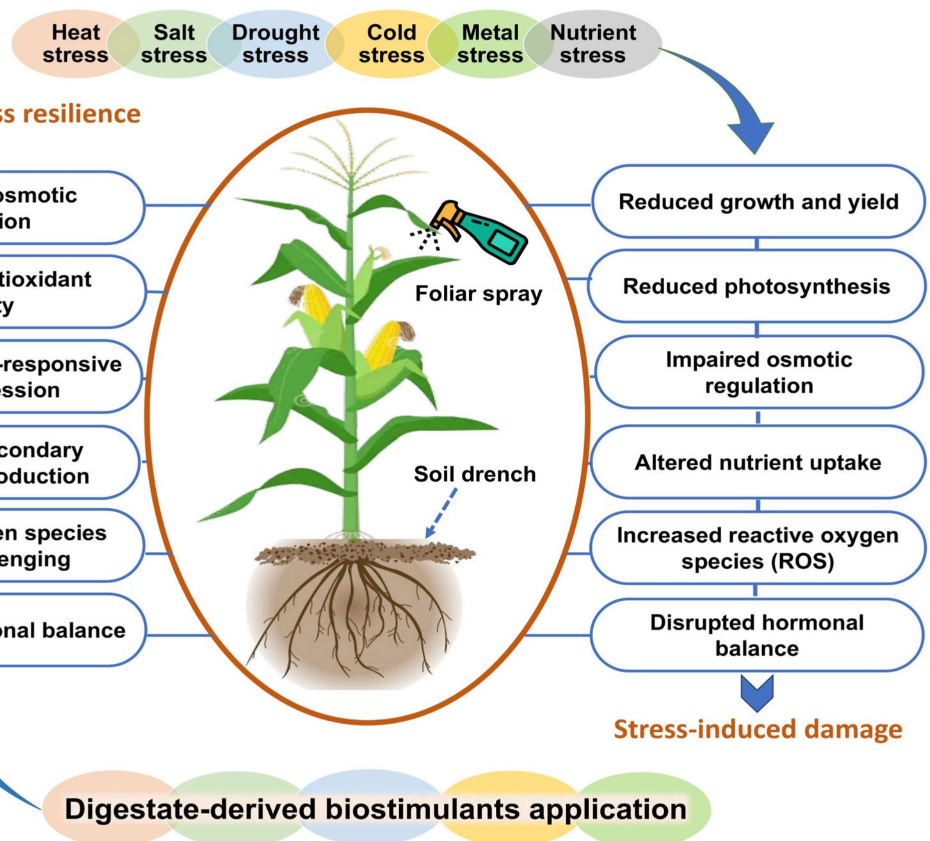

Fig. 4 Key mechanisms of digestate-derived biostimulants on abiotic stress tolerance in plants. Digestate-derived biostimulants enhance plant resilience to abiotic stresses such as heat, salt, drought, cold, metal toxicity, and nutrient deficiencies by modulating key physiological and molecular responses. These biostimulants improve osmotic regulation, boost antioxidant defences, activate stress-responsive genes, and stimulate secondary metabolite production, collectively aiding in reactive oxygen species scavenging and maintaining hormonal balance. Applied through foliar sprays or soil drenches, they mitigate stress-induced damage by preserving photosynthesis, nutrient uptake, and overall plant homeostasis. This demonstrates their potential as a sustainable solution for improving plant performance and stress tolerance in modern agricultural systems

digestate achieved complete removal of ammonium, phosphorus, and 17β-estradiol, while also significantly reducing zinc (62%), manganese (84%), cadmium (74%), and copper (99%) from diluted digestate [200]. Biosorption and bioaccumulation represent the predominant mechanisms through which algae facilitate the removal of heavy metals from anaerobic liquid digestate. Moreover, microalgae are highly efficient photosynthesizers, capable of capturing large amounts of CO_2 from the atmosphere or industrial emissions during their growth. By utilizing the CO_2 present in the air or flue gases, microalgae convert it into organic biomass through photosynthesis, effectively sequestering carbon [201, 202]. Thus, microalgae cultivation in liquid digestate and subsequent biostimulants production represent a promising avenue for advancing environmental sustainability.

Promoting the circular bioeconomy

In recent years, there has been a surge in the adoption of circular bioeconomy principles, driven by the need for sustainable resource management. The European Union, guided by directives promoting waste reduction, pollution control, and renewable resource efficiency, aligns with these principles [203–206]. Within this context, the production and application of plant biostimulants derived from digestate have gained prominence as an effective means to recover and produce valuable compounds from biomass waste, advancing the circular bioeconomy [11]. When biostimulants are used appropriately, the applications often notably increase yield, benefiting agriculture economically [15, 180, 207]. A life cycle assessment by Salomone et al. [208] revealed that reducing synthetic fertilizer production by means of applying biostimulants provides significant environmental benefits and leads to cost savings related to waste management.

Furthermore, developing microalgal biostimulants using residual nutrient sources such as liquid digestate provides unique opportunities for shaping circular economy platforms [31, 209]. As an example, the large-scale microalgae cultivation on digestate offers a circular bioeconomy solution by transforming organic waste into value-added products, closing nutrient loops, and promoting sustainable agricultural practices. This approach addresses waste management challenges and fosters resource efficiency, environmental sustainability, and agricultural resilience in a circular and interconnected manner. In doing so, it also creates fresh market opportunities for both the burgeoning biogas and biostimulants industries, aligning with the principles of the circular bioeconomy [210]. Thus, developing high-value digestate recycling streams, in a biorefinery approach, as depicted in Fig. 5, could result in generating additional revenues from these feedstocks, contributing to increased economic sustainability in the value chain; however, this requires further research and investment into such processes.

Digestate bioconversion provides a viable source of biostimulants for agricultural improvement. Challenges in converting digestates into biostimulants. Although these biostimulants have great potential, there are significant obstacles to their widespread implementation. One major obstacle to the effective utilization of biostimulants from bioconversion compost of solid digestate is the complex and variable composition of these organic materials. Many of the biostimulants found in bioconversion compost of solid digestate are humic substances, which include humic acids and fulvic acids. Often, these biostimulants are present as mixtures, and their identities remain unspecified [15]. Additionally, it exhibits substantial compositional variability, bioactive compounds, and microbial diversity arising from diverse feedstocks and digestion methods [211]. This compositional variability makes it challenging to standardize biostimulants production processes and affects the consistency and efficacy of the derived biostimulant products. Another significant challenge in utilizing biostimulants is the limited understanding of their modes of action. Furthermore, Yakhin et al. [15] highlighted that the stimulating effect of biostimulants on plants may not be attributed to a single component but rather to the synergistic effects of multiple substances. Consequently, the precise mechanisms through which biostimulants enhance plant growth and development remain elusive [170].

In addition, the utilization of digestate-derived microalgae as biostimulants in crop production is still in its nascent stage, necessitating further extensive research endeavours. The careful selection of suitable microalgae strains is of utmost significance in achieving high levels of biostimulants production. The persistent challenge lies in the quest for strains adept at efficiently utilizing liquid digestate nutrients to yield desired biostimulants. Moreover, maintaining an uncontaminated microalgae growth in liquid digestate poses significant difficulties, largely because of potential contamination from various bacteria. The careful regulation of unwanted microbial growth and competition is crucial to achieve the desired outcomes in biostimulants manufacturing. To this end, it is imperative to undertake additional research to optimize the microalgae cultivation process within liquid digestates, encompassing critical parameters such as nutrient concentration, pH modulation, temperature control, light intensity optimization, and the meticulous selection of cultivation systems.

Another significant impediment to the extensive adoption of biostimulants derived from microalgae is the need for economically viable manufacturing methods. Presently, the market for microalgae-derived biostimulants predominantly features a limited assortment of products, with the majority categorized as "primary products” such as extracts indicative of their relatively rudimentary or lower quality nature. This categorization is primarily attributed to limitations in the production of biostimulants characterized by high efficacy and exceptional purity [212]. Consequently, the absence of cost-effective manufacturing technologies restricts the broader utilization of microalgae-derived biostimulants in modern agricultural practices.

A further challenge is the lack of comprehensive understanding regarding the mode of action of biostimulants and their interactions with soil and microbes in the field conditions, which is exacerbated by a scarcity of long-term data on their impact on soil quality, crop productivity, and related factors [11]. Moreover, it is crucial to acknowledge that the responses of plants to biostimulants can be significantly influenced by climate change, encompassing complex interactions above- and belowground within the growing environment, as well as the diversity of organisms present in terrestrial ecosystems. Consequently, the practical application of biostimulants must demonstrate effectiveness under varying field conditions [167, 213, 214]. Notably, several studies have reported inconsistent outcomes when attempting to utilize biostimulants in field settings, underscoring the multifaceted nature of interactions involving plants, symbiotic microbial species, the ecological impacts of plant-associated soil microbes and soil composition, as well as the dynamic metabolic processes within plants [213, 215, 216]. Thus, while numerous investigations have substantiated the advantages of digestate-derived biostimulants in agricultural production, there remains a pressing need to establish a more robust scientific framework.

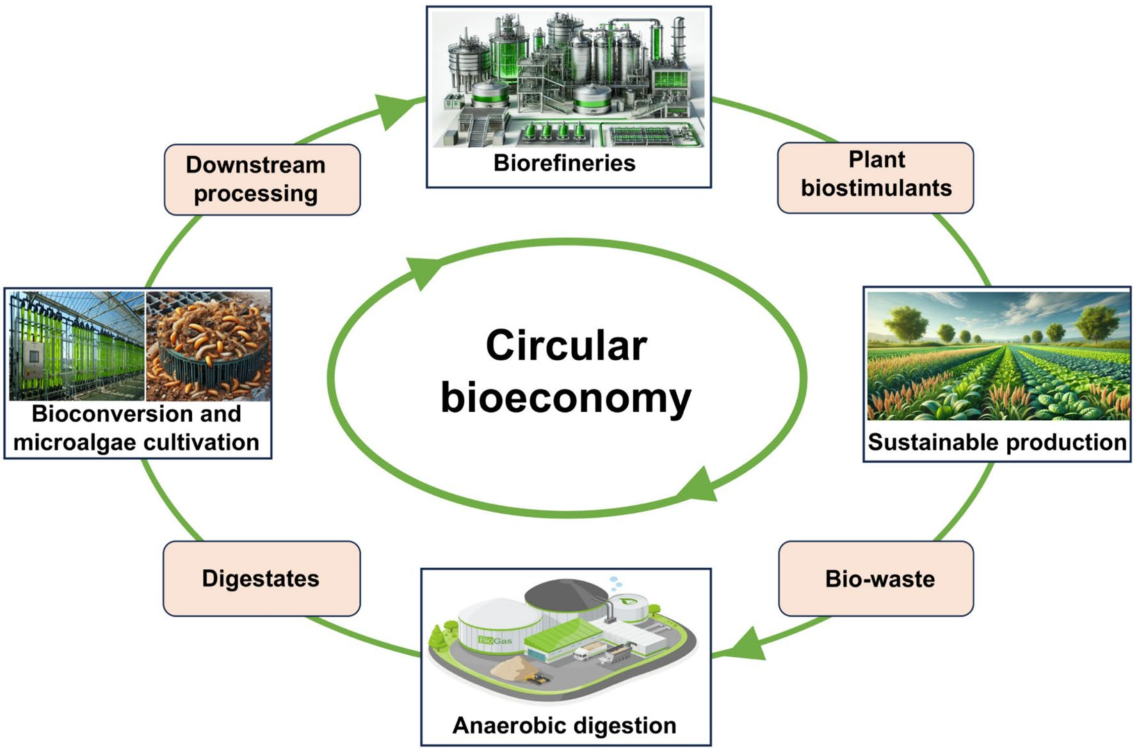

Fig. 5 Bioeconomy context of biostimulants production through resource selection and digestate recycling. Organic waste streams are converted into high-value bioproducts through a closed-loop circular bioeconomy where bio-waste is transformed into digestates, which are then subjected to bioconversion processes to produce bioactive compounds. These compounds are refined into high-value plant biostimulants through downstream processing in biorefineries, which are then applied to enhance agricultural productivity and sustainability. This system exemplifies the principles of the circular bioeconomy by ensuring complete recycling of waste into beneficial products, optimizing resource use, and minimizing environmental impact, while simultaneously supporting resilient, sustainable agricultural systems

Designing the next generation of biostimulants

Synergistic interactions between different types of digestate-derived biostimulants have the potential to maximize their effectiveness in agriculture. This section explores the possible synergisms between digestate-derived biostimulants, highlighting their combined improved effectiveness for soil health and plant performance. Combining biostimulants may result in three plausible outcomes: additive, antagonistic, and synergistic effects based on their interactive bio-stimulatory actions and mechanisms [207]. Firstly, for additive effects, the combined effects exerted by biostimulants equal the sum of their individual effects. Secondly, for antagonistic effects, the overall effect exerted by biostimulants delivers less than the additive effects. Lastly, synergistic effects are observed when the cumulative effects of biostimulants exceeded their additive effects, which is ultimately the preferred outcome.

In recent years, advances in biochemical, genomic, and transcriptomic tools have facilitated significant progress in understanding biostimulants' mode of actions [19, 217]. These technological advances have opened new avenues for the biostimulants industry, encouraging the exploration of more effective and reliable formulations by combining various biostimulants. Current advances in biostimulant research reveal a trend in which the combination of biostimulants, as compared to their individual application, has demonstrated the capacity to produce more substantial enhancements in plant growth, yield, and quality [218]. Moreover, this combined strategy has the potential to supplement or even replace conventional agrochemicals without compromising yield or ecological sustainability [219].

When judiciously chosen and applied, these biostimulant combinations have proven effective in exerting desired effects on plants confronting a multitude of abiotic constraints, including nutrient limitations, drought, and salinity [177]. For instance, Sani and colleagues [220] demonstrated that the combined application of *Trichoderma*-based microbial biostimulants and algal extracts synergistically enhanced the growth and nutritional quality of organically grown tomatoes. This synergy was attributed to increased soil fertility, improved chlorophyll biosynthesis, and higher photochemical activity in photosystem II, resulting in superior leaf tissue nutritional status. Rouphael and colleagues [221] found that the application of protein hydrolysates and seaweed extracts enhances lettuce's salinity tolerance, with PHs stimulating the accumulation of glucosinolates and phytoalexin precursors while seaweed extracts reduce the accumulation of secondary metabolites, potentially indicating synergistic effects on various processes. Furthermore, Poveda and Eugui [222] evaluated the synergistic potential of *Trichoderma*, along with beneficial bacteria such as *Bacillus* and *Pseudomonas*, as microbial bio-inoculants in agricultural production. Their findings revealed a remarkable combination that acts as a powerful plant growth promoter and exerting biocontrol effects through diverse synergistic mechanisms.

Commercializing digestate-derived biostimulants

For a successful route to the commercialization of digestate-derived biostimulants, the aspects summarized in Fig. 6 need to be considered carefully. First, research should prioritize the recovery and purification of biostimulant products through optimized downstream processing of digestate. Second, identifying and refining efficient extraction methods to obtain biostimulants with high bioactive metabolite content is essential. Third, extensive analytical efforts are needed to expand metabolome coverage, thereby facilitating the discovery of novel precursors or bioactive compounds for biostimulant application. Fourth, a comprehensive identification and quantification of active ingredients in biostimulant formulations, coupled with metabolomic profiling, is crucial for elucidating their modes of action. Fifth, a deeper understanding of the genetic and molecular biosynthetic pathways within plant cells, as well as their interactions with the rhizosphere microbiome, will enable more targeted and effective applications. Sixth, establishing rigorous standards for purity, quality, and safety is vital to ensure the reliability and efficacy of these products in agricultural contexts. Finally, advancing biorefinery concepts and promoting sustainable circular economy practices, including the integration of digestate recycling, are fundamental to realizing the full potential of digestate-derived biostimulants.

In the context of field-based studies and process scale-up, research should focus on several key aspects to enhance the efficacy and adoption of biostimulants. First, validating the optimal application methods, concentrations, timing, duration of biostimulant effects, and dosage frequencies is essential for maximizing biostimulant activity across various crops. Second, understanding the influence of seasonal changes, co-applications, and interactions with other components, as well as variations in crop types, soil conditions, stress factors, and the composition of the rhizosphere and endosphere microbiomes, is critical for effective biostimulant performance. Third, employing high-throughput sequencing, phenotyping, and omics methodologies is necessary to characterize and drive the development of innovative biostimulants. This approach facilitates the discovery of mechanisms through which specific bioactive compounds or combinations affect plant performance and allows researchers to explore synergistic or antagonistic interactions between biostimulants, crop varieties, and microbiomes, ultimately enabling the customization of formulations. Fourth, exploring potential co-applications or blends, such as combining microalgal biostimulants with microbial biostimulants, macroalgae extracts, or commercial fertilizers, is vital to identify synergistic interactions and complementary functionalities that could enhance overall biostimulant efficacy. Fifth, gaining a comprehensive understanding of the environmental and economic impacts through life cycle assessment and techno-economic assessment tools, alongside rigorous environmental safety assessments, is essential to move beyond the simple view of biostimulants as "eco-friendly alternatives to conventional fertilizers". Sixth, ensuring regulatory compliance and alignment with industry standards will foster responsible usage, innovation, and market acceptance of biostimulant products. Lastly, translating laboratory findings to field applications must be thoroughly validated to support commercialization efforts.

Advancing biostimulant development further requires a strategic balance among production control, identification of target consumer segments, regulatory adherence, and compliance with safety and organic certification standards. This integrated approach, within a secure and standardized framework, is crucial for achieving profitability and competitiveness in the evolving market landscape. To support this, research should concentrate on the following: establishing policies to address regulatory challenges, intellectual property rights, and patent issues, with a particular focus on meeting organic certification, eco-labelling standards, and safety requirements for biostimulants derived from digestate streams. Additionally, instituting a standardized and secure framework can build market credibility and instil confidence among both producers and consumers.

Conclusion

The utilization of anaerobic digestates to produce biostimulants offers a promising avenue for more sustainable organic waste management and the advancement of highly productive and robust cultivation systems in agriculture. These digestate-derived biostimulants include humic substances, protein hydrolysates, phytohormones, betaines, microalgal polysaccharides and other bioactive metabolites. They function through multiple mechanisms,

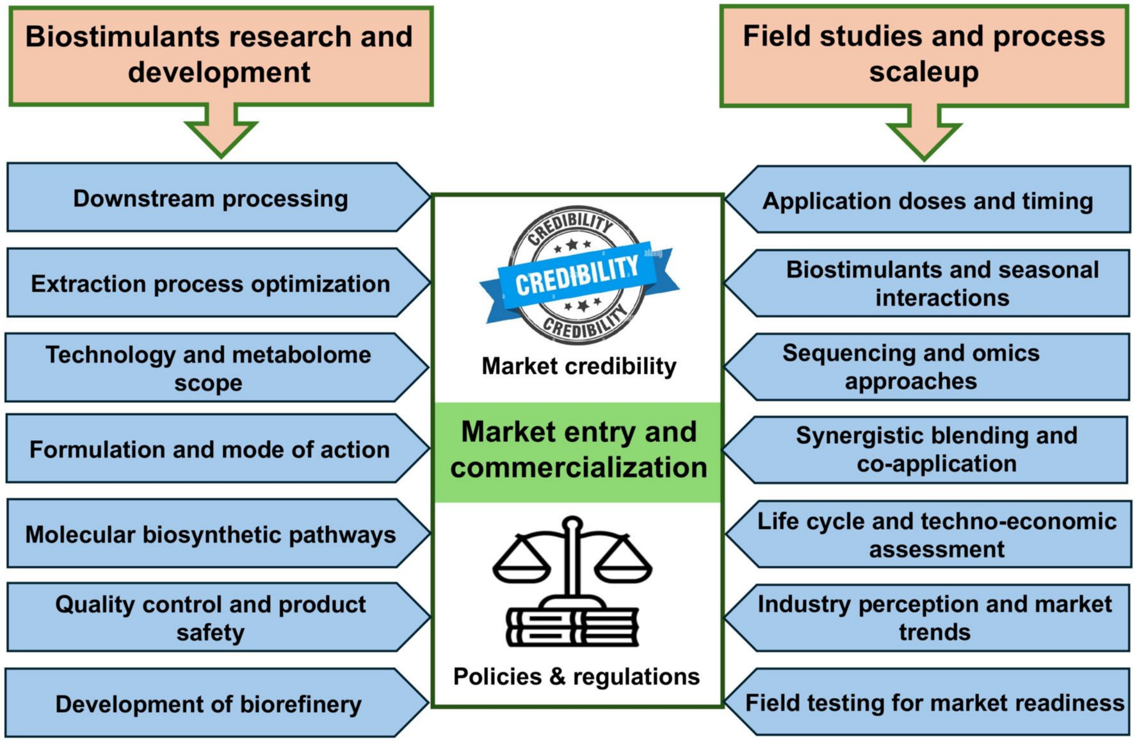

Fig. 6 Pathway and key aspects for the development and market integration of digestate-derived biostimulants. Research and development focus on optimizing extraction processes, exploring molecular biosynthetic pathways, and ensuring product safety and formulation efficacy. Parallel field studies emphasize application timing, dose optimization, and understanding biostimulant-environment interactions through omics approaches and synergistic applications. The successful commercialization of biostimulants hinges on market credibility, driven by regulatory compliance and field-tested validation. This integrative approach ensures that biostimulants derived from digestate can meet market demands while supporting sustainable agricultural systems

including enhanced nutrient uptake, root growth promotion, stress tolerance induction, and regulation of endogenous plant hormone levels and metabolic signalling pathways leading to enhanced growth and resilience. The presence of microorganisms in digestate bioconversion composts further enhances soil health by promoting nutrient cycling and rhizosphere microbial dynamics. While biostimulants offer potential benefits regarding crop yield enhancement, and climate-resilient agriculture through improved soil health, understanding their underlying mode of action remains a significant challenge. Additionally, addressing the variability in digestate composition from different feedstocks to ensure consistent bio-stimulatory effects in biostimulant products poses another bottleneck for wider adoption among growers. Moving forward, the development of molecular tools, including high-throughput phenotyping and -omics approaches, is anticipated to aid in identifying and isolating novel compounds, exploring synergistic interactions, and elucidating the mechanisms behind digestate-derived biostimulants action. Additionally, exploring the potential synergistic and complementary functionalities in co-application scenarios holds promise for tailored formulations and enhanced biostimulant functionality. Furthermore, successful biostimulants market entry and commercialization must consider several vital factors such as the strategic balance between production control, target consumer niches, regulatory frameworks, safety, and organic certification standards. As the industry evolves, employing a diverse array of multi-disciplinary technologies, including microbiology, biochemistry, and agronomy will be instrumental in realizing the full potential of biostimulants from digestates and fostering a more sustainable and climate-resilient agricultural future.

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Authors' contributions Md.NHS prepared the first draft of the manuscript and contributed to conceptualization, investigation, visualization, review and editing. MA participated in writing original draft and visualization. K-JB contributed through review, editing, and supervision. SC provided support in review, editing, and supervision. TP was involved in review, editing, supervision, and funding acquisition. JWHY contributed to review and editing, funding acquisition, validation, and supervision. All authors read and approved the final version of the manuscript.

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Declarations

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