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Review

Breaking New Ground: Exploring the Promising Role of Solid-State Fermentation in Harnessing Natural Biostimulants for Sustainable Agriculture

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Abstract: Agriculture has been experiencing a difficult situation because of limiting factors in its production processes. Natural biostimulants (NBs) have emerged as a novel alternative. This study reviews NBs produced through solid-state fermentation (SSF) from organic waste, focusing on processes and production methods. The aim is to highlight their potential for improving agricultural productivity and promoting sustainable agriculture. Through a literature review, the effects of NBs on crops were summarized, along with the challenges associated with their production and application. The importance of standardizing production processes, optimizing fermentation conditions, and assessing their effects on different crops is emphasized. Furthermore, future research areas are introduced, such as enhancing production efficiency and evaluating the effectiveness of SSF-produced NBs in different agricultural systems. In conclusion, SSF-produced NBs offer a promising alternative for sustainable agriculture, but further research and development are needed to maximize their efficacy and to enable large-scale implementation.

Keywords: natural biostimulant; solid-state fermentation; organic waste; sustainable agriculture; crop improvement

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1. Introduction

One of the main challenges in agriculture is achieving global zero hunger [1]. Therefore, sustainable agriculture is a viable method to ensure food security. In this regard, the Food and Agriculture Organization of the United Nations (FAO) envisions providing nutritious and accessible food for all while preserving natural resources to meet current and future needs. Sustainable agriculture also aims to benefit producers in terms of economic development [1]. In conventional agriculture, reducing the intensive use of agrochemicals is a significant challenge that negatively impacts soil health, water scarcity, and biodiversity [2]. In this context, natural biostimulants (NBs) have emerged as alternatives to sustainable agriculture. NBs are derived from products such as microorganisms, plant extracts, and seaweed extracts and can be classified into three main groups based on their source and content: humic substances (HS), hormone-containing products (HCP), and amino-acid-containing products (AACP). HCP, such as seaweed extracts, contain various active substances for plant growth, including auxins, cytokinins, and their derivatives [3]. These products contain biologically active compounds that stimulate plant physiological processes and promote growth, development, and resistance to biotic and abiotic stresses [4–7]. NBs offer significant advantages because they are derived from natural sources, such as waste materials, plant extracts, and microorganisms [8,9], making them more environmentally sustainable than chemical products based on synthetic compounds. Furthermore, NBs are generally safer for the environment and human health than chemical products, which can be harmful [10]. NBs also have the potential to promote beneficial interactions with soil microorganisms, unlike chemical products that lack this capacity [11]. Additionally, NBs can serve as an easier alternative to chemicals in order to comply with regulations and restrictions in many countries [6,12,13]. Given these issues, NBs present themselves as a promising alternative in agriculture.

Various production methods exist, including solid-state fermentation (SSF), a technology conducted in the absence or near absence of free water, allowing the use of solid materials as substrates for enhanced biotransformation. SSF has been reported as a promising eco-technology for the production of bio-based products, and studies have demonstrated the successful pilot-scale production of NBs using plant biomass as a support and carbon source for different microorganisms. These production processes are performed under controlled conditions, including temperature, humidity, and airflow, to optimize NB synthesis [14,15]. Furthermore, the utilization of organic waste as a substrate in the SSF process has gained attention, primarily involving various solid biodegradable materials derived from agricultural and forestry byproducts and waste [16]. NBs obtained through SSF have shown biostimulant effects on crop development, including physical parameters such as germination, growth, stem length, leaf count, root dry weight, leaf area, biomass production, macronutrients, and micronutrients [17]. They have also demonstrated positive effects on root development in forest species [18]. Therefore, NBs produced through SSF represent an emerging alternative to the limitations of conventional biostimulants, including their negative impact on agricultural sustainability, the need to reduce the impact of waste on the environment, and the desire to limit the use of synthetic compounds in agriculture [19].

This review addresses the production of NBs through SSF using organic waste as a promising approach for sustainable agriculture. Furthermore, these NBs have the potential to enhance plant growth and development while reducing reliance on conventional chemical products. To achieve this, the existing literature was reviewed to assess the effectiveness and limitations of NB production through SSF.

2. Materials and Methods

Methodology

This review article involved the selection of scientific articles from the following scientific databases: SpringerLink (https://link.springer.com/, accessed on 25 May 2023), Science Direct (https://www.sciencedirect.com/, accessed on 25 May 2023), Wiley (https://onlinelibrary.wiley.com, accessed on 25 May 2023), ProQuest (https://www.proquest.com/, accessed on 25 May 2023), Patent Inspiration (https://www.patentinspiration.com/, accessed on 25 May 2023), and Web of Science (https://www.webofscience.com/, accessed on 25 May 2023). Boolean operators (AND and OR) were used to obtain more accurate results. The following keywords were used: "solid state fermentation and biostimulant", "solid state fermentation and auxins", "solid state fermentation and biostimulant name". Literature from the past 30 years was included in the article review.

Articles were selected based on the following inclusion criteria: relevance of the publication to the topic and selected years. The following criteria were considered: type of NB, substrate, microorganisms, optimal conditions, and effects on crops. We aimed to address these research questions by collecting and analyzing relevant studies, considering the latest trends in NB production through SSF using organic waste.

3. Relevant Sections

3.1. Definition and Types of Biostimulants

NBs are derived from natural sources such as microorganisms, plant residues, and seaweed, among others [20]. These products contain biologically active compounds that stimulate plant physiological processes, promoting plant growth, development, and resistance to biotic and abiotic stresses [10]. However, biostimulants include a wide range of compounds, as highlighted by the European Biostimulants Industry Council (EBIC) and the Biological Products Industry Alliance (BPIA) [14]. The EBIC defines plant biostimulants as substances or microorganisms that stimulate natural processes to enhance nutrient uptake, efficiency, stress tolerance, and crop quality. They do not a have direct pesticidal action and are not regulated by pesticide laws. BPIA defines biostimulants as diverse materials that improve crop vigour, quality, yield, and tolerance to abiotic stresses by facilitating nutrient uptake, enhancing soil microorganism development, and stimulating root growth to increase water-use efficiency [12,13]. This growth is in line with an increase in scientific support for the use of biostimulants as agricultural inputs for various plant species [21].

Currently, there are various types of NBs, including those produced by SSF, which can serve as a starting point for future research (Table 1).

Table 1. Types of NBs, mode of action, and effects produced by SSF.

Natural Product	Type of NB	Molecules Present	Action Mode	Biostimulant Effect	SSF-Relevant Origin	Refs.
Hormone-Containing Products (HCP)	Auxins	3-indoleacetic Acid (IAA)	Promotes cell elongation	Stimulates cell elongation and rooting	Produced by SSF	[22,23]
		Indole Propionic Acid (AIP)	Promotes vegetative growth and cell division	Stimulates growth, flowering, and rooting in plants	Not produced by SSF	[24,25]
	Cytokinins	Zeatin	Stimulates cell division and vegetative growth	Promotes growth and development of plants	Not produced by SSF	[26–28]
		Kinetin	Stimulates cell division and vegetative growth	Improves the quality of the crops, increasing the size and weight of the fruits	Produced by SSF and vermicompost	[29–31]
	Abscisic Acid (ABA)	ABA	Regulates stress responses and plant development	Improves stress tolerance and fruit ripening	Produced by SSF	[32,33]
	Gibberellins	Gibberellin A3 (GA3)	Stimulates growth and vigor in plants	Inducts germination and flowering	Produced by SSF	[34–36]
		Gibberellin A4 (GA4)	Promotes plant growth and development	Stimulates germination, development of lateral shoots, and flowering	Produced by SSF	[37,38]
Seaweed Extract (AM)	AM	Alginic Acids	Improves nutrient absorption and stimulates enzyme activity	Increases growth and resistance to abiotic stress	Produced by SSF	[39–41]
		Fucoidan	Improves the defense mechanisms of plants	Increases resistance to abiotic stress	Produced by SSF	[42–45]
		Oligosaccharides	Stimulates physiological responses in plants	Improves immune response and growth	Produced by SSF	[46–49]
Humic Substances	Humic and Fulvic Acids (AHF)	Humic Acids	Improves soil structure and nutrient availability	Stimulates root growth and nutrient absorption	Produced by SSF	[50–53]
		Humic Acids	Stimulates plant growth and development	Improves nutrient uptake and stress resistance.	Produced by SSF	[54,55]
Amino-Acid-Containing Products (AACP)	Amino Acids	L-proline	Regulates plant stress and development	Enhances stress tolerance and resistance	Produced by SSF	[56–58]
	Peptides	Low Molecular Weight Peptides	Stimulates plant growth and development	Improves plant nutrition and growth	Produced by SSF	[59–61]
Other NBs	Siderophores	Siderophores	Binds to Fe and is solubilized	Improves absorption and mobilization of Fe	Produced by SSF	[62–64]
	Chitosan Fungal	Chitosan Fungal	Promotes plant growth, cell division, increases enzyme activity, and improves nutrient transport	Presents biostimulant activity in seed germination	Produced by SSF	[65,66]

3.2. Advantages of Natural Biostimulants over Conventional Ones

In this regard, NBs obtained through SSF have emerged as an alternative to conventional biostimulants, primarily because of their positive impact on agricultural sustainability, reduced environmental waste, and limited use of synthetic compounds in agriculture [46].

NBs obtained by SSF from organic waste are gaining interest because of their numerous advantages over conventionally synthesized biostimulants [47]. This article reviews and compares the advantages of NBs in terms of effectiveness, safety, sustainability, and environmental benefits. Among these advantages, the following can be highlighted.

3.2.1. Sustainability and Environmental Impact

The importance of NBs as a sustainable option in agriculture lies in their renewable origin and lower environmental impact than chemical biostimulants [21].

Generally, the use of NBs has a positive environmental impact [19,48,49]. They can help to reduce or rationalize the amount of synthetic fertilizers and pesticides needed to grow plants [67–69]. For example, some NBs can have a positive effect on microbial communities in the soil and can be beneficial for agricultural practices [11]. In terms of environmental impact, NBs extracted from microorganisms are non-toxic and do not pollute the environment [70,71]. In addition, because they are obtained from natural sources, their production is more sustainable than that of chemical biostimulants.

3.2.2. Security

In contrast to the risks associated with the chemicals used in chemical biostimulants, NBs tend to be safer for both the environment and human health [72].

3.2.3. Broad Spectrum of Activity

NBs have a wide spectrum of activities, which implies multiple benefits for plants in terms of growth, nutrient absorption, stress resistance, flowering, and fruiting quality [20,73].

3.2.4. Positive Interactions

NBs promote beneficial interactions with soil microorganisms, improving soil health and favoring more balanced and productive agricultural systems [49,74].

3.2.5. Regulatory Compliance

NBs offer an easier option for complying with government regulations and restrictions on the use of chemicals in agriculture, which has become more relevant in many countries [6].

3.3. Production Processes of NBs by SSF

Thus, SSF is a promising method for NBs production. SSF produces a variety of bioactive products that promote plant growth, development, and responses to abiotic and biotic stress conditions [75,76]. In this chapter, the processes used to obtain natural biostimulants through SSF were explored, highlighting their importance and efficacy in sustainable agriculture.

3.3.1. Substrate Selection in NB Production by SSF

The appropriate choice of substrates is a crucial step in the production of NBs by SSF [77]. Substrates provide a source of nutrients, energy, and bioactive compounds for microorganisms during fermentation. [78]. The most commonly used substrates in SSF include agricultural residues, agro-industrial waste, food industry by-products, and lignocellulosic materials [16]. These substrates are rich in nutrients and can be degraded by microorganisms, allowing the production of beneficial metabolites [79].

3.3.2. Substrate Pretreatment

Pretreatment of substrates is necessary to improve their composition and nutrient availability. Pretreatment may involve steps such as crushing, grinding, sieving, pH adjustment, sterilization, and addition of nutritional agents [75,80,81]. These steps aim to optimize the conditions for microbial growth and production of desired metabolites [82]. Pretreatment can also facilitate the degradation of substrates and increase fermentation efficiency [83].

3.3.3. Microorganisms for NB Production by SSF and Inoculation

Microorganisms play a fundamental role in the production of NBs by SSF, as they are responsible for substrate degradation and synthesis of bioactive metabolites [84]. In this section, we will focus on the different microorganisms used in this process and their relevance to NB production.

Examples of microorganisms used in SSF for NB production include bacteria, fungi, and yeasts. Each type of microorganism possesses specific characteristics that can influence biostimulant production.

The inoculation of microorganisms is a crucial step in the production of NBs by SSF [18]. Beneficial microorganism strains such as bacteria, fungi, and yeast are selected for their ability to degrade substrates and produce bioactive metabolites. These microorganisms were pre-cultivated under optimal conditions and then inoculated into substrates to initiate SSF [78,84]. The choice of suitable microorganisms and their interactions during SSF influence the composition and final quality of the biostimulant [18].

3.3.4. Control of SSF Conditions

Control of SSF conditions is essential for obtaining high-quality biostimulants through SSF. Parameters such as the temperature, humidity, pH, C/N ratio, moisture content, and process duration must be monitored and adjusted accordingly. These conditions affect the growth and metabolism of microorganisms [15,18]. The precise control of SSF conditions ensures the optimization and quality of the biostimulant.

The production of natural biostimulants through SSF involves the selection of suitable substrates, pretreatment of substrates, inoculation of microorganisms, and control of SSF conditions. These processes are crucial for obtaining high-quality NBs that can promote plant growth.

3.3.5. SSF Bioreactors in NB Production

The use of SSF bioreactors has proven to be a promising technique for improving NB production. These systems allow for better control of fermentation conditions and higher efficiency in obtaining high-quality biostimulants [15].

SSF bioreactors can be designed to maintain optimal cultivation conditions, including temperature, humidity, aeration, and water content [85]. The appropriate selection of the bioreactor depends on various factors, such as the type of microorganism, substrate used, and desired production scale [86]. Common types of SSF bioreactors include fixed-bed, fluidized-bed, and packed-bed bioreactors [79]. The implementation of SSF bioreactors in NB production represents a significant improvement in the efficiency and quality of biostimulants, contributing to a more sustainable and productive agriculture [87,88]. Table 2 presents examples of substrates commonly used in the production of NBs by SSF, together with their characteristics and advantages, microorganism selection, production mode, and bioreactor type.

Table 2. Comparison of substrates, microorganism selection, production mode, and ioreactor type in NB production by SSF.

Substrate	Characteristics and Advantages of Substrate	Microorganism Selection	Production Mode	Bioreactor Type	Refs.
Crop Residues	Abundant local availability, nutrient source, and microorganism support	Bacteria, Fungi	Batch, Continuous, Fed-Batch	Fixed-Bed, Packed-Bed	[89–91]
Agroindustrial Waste	Waste valorization and reduced environmental impact	Filamentous Fungi	Batch, Continuous	Fluidized-Bed, Packed-Bed	[47,92]
Food Residues	Rich in nutrients and organic matter, avoids food waste	Bacteria, Filamentous Fungi	Batch, Fed-Batch	Fixed-Bed, Packed-Bed	[37,93]
Plant Residues	High content of bioactive compounds and phytohormones	Bacteria, Filamentous Fungi	Batch, Continuous	Fluidized-Bed, Packed-Bed	[94,95]
Algal Biomass	Rich in bioactive compounds and auxins	Microalgae	Batch, Fed-Batch	Bubble-Column	[96,97]
Wood Residues	Sustainable source with lignocellulosic content	Filamentous Fungi	Fed-Batch, Continuous	Fluidized-Bed, Packed-Bed	[98,99]
Residual Sludge	Reduces waste volume and provides rich source of nutrients	Bacteria, Filamentous Fungi	Batch, Continuous	Plug-Flow, Packed-Bed	[100,101]
Fishery Waste	Utilization of waste from the fishing industry	Filamentous Fungi	Batch, Continuous	Packed-Bed	[102,103]
Brewery Waste	Valorization of waste from brewing processes	Filamentous Fungi	Continuous	Packed-Bed	[104,105]
Citrus Waste	Abundant source of bioactive compounds and antioxidants	Filamentous Fungi	Batch, Fed-Batch	Fixed-Bed, Packed-Bed	[33,106]
Coffee Residues	Rich in bioactive compounds and promotes soil health	Filamentous Fungi	Batch, Continuous	Packed-Bed	[107]
Rice Husk	Rich in organic matter and bioactive substances	Filamentous Fungi	Fed-Batch, Continuous	Packed-Bed	[108]

4. Methods of NB Production

In this section, the production methods used to obtain NBs through SSF are addressed. The type of biostimulant, microorganisms used in this process, and the optimal conditions of SSF for its production will be described.

4.1. Microorganisms Used in NB Production

In the production of NBs through SSF, various beneficial microorganisms play key roles in substrate degradation and the synthesis of metabolites. Examples of microorganisms used include bacteria, fungi, and yeast. Each type of microorganism possesses specific characteristics that can influence biostimulant production. See Table 2.

4.2. Characteristics of SSF for NB Production

SSF is used to produce natural biostimulants. In this process, microorganisms are cultivated on solid substrates, such as agricultural residues or by-products of the food industry. During fermentation, microorganisms secrete enzymes and bioactive metabolites that transform the compounds present in the substrate into forms that are readily assimilated by plants [18].

The biological activity determines the production of NBs and warrants particular attention in future research. Table 3 presents examples of substrate microorganisms used to obtain different natural biostimulants (NBs) through SSF.

Table 3. Methods of NB production by SSF.

NB	Substrate	Microorganism	Pretreatment			Optimal SSF Conditions		Effect of NBs on Crop	Refs.
			Trituration	pH	Sterilization	Moisture %	Temperature °C		
IAA	Pruning Waste + Grass	*Trichoderma harzianum*	1 cm	6.8	2 times	74	25		[15]
IAA	Yuca Bagasse Soy Bran Wheat Bran Sorghum Dried Distiller's Grains Corn Dried Distiller's Grains	*Aspergillus flavipes* *Aspergillus ustus* *Bacillus subtilis* *Bacillus megaterium* *Bacillus amyloliquefaciens* *Trichoderma atroviride* *Trichoderma koningii* *Trichoderma harzianum*	0.5, 1.0 y > 1.0 mm			50	Room Temperature	Clon IPB2 *Eucalyptus grandis* and *Eucalyptus urophylla* Increasing Rooting	[14,18]
Kinetin	Cow Dung + Leaf Litter	*Selenomonas ruminantium*	2–5 mm	6.9		70–75	25 ± 3		[29]
ABA	Millet Rice	*Botrytis cinerea*	Millet and Rice		1 time		26.5–25.5		[32]
GA3	Rice Bran	*Gibberella fujikuroi*			50 °C	65.95%	28 ± 2		[109]
GA3	Corn Cob Residues	*Aspergillus niger*		5.1		24%			[110]
GA3	Citric Pulp	*Fusarium moniliforme* LPB03 + *Gibberella fujikuroi*		5.5–5.8		75	29		[91]
Alginic Acids	Apple Peels	*Azotobacter vinelandii*, NRRL-14641	0.1 mm	7	60 °C	70	37.5		[39]
Alginic Acids	Sargassum Macroalgae	*Cunninghamella echinulate* *Aspergillus niger* *Penicillium oxalicum*		7–8.5	1 time 121 °C	65–75	28–30		[40]
Fucoida	Seaweed *Fucus Vesiculosus*	*Aspergillus niger* *Mucor sp*				80	30		[42]
Oligosaccharides	Soybean Meal	-					Room Temperature	Effect on Germination	[111]
Chitin Oligosaccharides	Powder of Molting of Mealworms	*Talaromyces allahabadensis Hi-4* *Talaromyces funiculosus*		6			40		[112]

Table 3. *Cont.*

NB	Substrate	Microorganism	Pretreatment			Optimal SSF Conditions		Effect of NBs on Crop	Refs.
			Trituration	pH	Sterilization	Moisture %	Temperature °C		
Humic Acid	Oil Palm Empty Fruit Bunch	*Trichoderma reesei*		6		64–72	30		[50,113]
Fulvic Acid	Sugarcane Bagasse	*Trichoderma* Sp.				70	20		[114]
L-proline	Wheat Straw Ice Straw Wheat Bran Corn Cob Corn Stover	*Fomitopsis* sp.	Small Pieces	5.5			25–30		[56]
Low Molecular Weight Peptides	Chickpeas	*Bacillus subtilis*							[60]
Siderophores	Soybean Protein Meal	*Lactobacillus plantarum*					37		[115]
Chitosan Fungal	Sweet Potato	*Gongronella butleri USDB 0201*					28		[66]

4.3. Effect of the NBs on Crops

As detailed in previous chapters, NBs have a significant impact on crop growth, development, and yield. The following are examples of observed effects on different aspects of crop production, supported by scientific studies.

4.3.1. Improvement of Plant Growth and Development

The application of NBs promotes root growth, increases plant biomass, improves plant architecture, and enhances seed germination and seedling emergence. These effects are attributed to the presence of specific molecules in NBs, such as low molecular weight peptides, gibberellic acid (GA3), and indole-3-acetic acid (IAA) [116–119].

Table 4 summarizes the effects of NBs on crop growth and development.

Table 4. Effect of NBs on improving plant growth and development.

Crop	NB Type	Effect	Scale	Refs.
Arabidopsis thaliana	Low Molecular Weight Peptides	Increase in plant biomass	Laboratory	[120]
Sesame	GA3	Improvement of plant architecture	Laboratory	[121]
Rice	GA3	Improvement of plant architecture	Laboratory	[122]
Tomato Pepper Seed Arabidopsis Orchid	IAA	Promotion of seed germination and seedling emergence	Greenhouse Laboratory	[17,123,124]

4.3.2. Increased Resistance to Adverse Conditions

In addition to improving plant growth and development, NBs also enhance the resilience of crops against adverse conditions. It has been observed that certain molecules present in NBs, such as ABA and seaweed polysaccharides, contribute to increased tolerance to abiotic stress, enhanced disease and pest resistance, and protection against oxidative stress [125,126].

Table 5 summarizes some NBs and their effects on resistance to adverse conditions.

Table 5. Effect of NBs on resistance to adverse conditions.

Crop	NB Type	Effect	Scale	Refs.
Orange Tobacco Corn	ABA	Abiotic stress tolerance	Laboratory	[127–129]
Strawberry Bean Vine Cucumber	Seaweed Polysaccharides	Resistance to diseases and pests	Field	[130–133]

4.3.3. Effect of NBs on Improving Crop Quality

In this section, we will explore scientific studies that have investigated the influence of different NBs on improving the quality of various crops. Aspects such as nutritional content, physical appearance, shelf life, and resistance to stress will be addressed (Table 6). These findings provide a solid foundation for understanding the potential of NBs for enhancing crop quality and open new perspectives for their application in sustainable agriculture.

Table 6. Effect of NBs on enhancing resistance to adverse conditions.

Crop	NB Type	Effect	Scale	Refs.
Gerbera Tectona Grandis Peas Yarrow	Humic Acid	Increased nutrient concentration	Greenhouse	[134–137]
Tomato Apple	Amino Acids	Improved organoleptic quality	Greenhouse	[138–140]
Soy Petunia Flowers Lettuce	Cytokinins	Delayed tissue senescence	Greenhouse	[141–143]

4.3.4. Optimization of Nutrient Use Efficiency

In this section, we focus on optimizing nutrient use efficiency in crops through the use of NBs. Nutrient use efficiency is a key factor in agricultural production as it directly influences the absorption, assimilation, and utilization of nutrients by plants. NBs have been demonstrated to be an effective tool for improving this efficiency and maximizing crop yield. Table 7 presents evidence of how NBs enhance nutrient use efficiency.

Table 7. Effect of NBs on optimal nutrient use.

Crop	NB Type	Effect	Scale	Refs.
Tomato Strawberries Peanut	Alginic Acids	Improvement of nutrient availability in the soil	Greenhouse	[144–146]
French Marigold	Oligosaccharides	Reduced nutrient losses	Greenhouse	[147,148]

4.3.5. Effect NBs on Agricultural Productivity

NBs are a promising tool for enhancing crop efficiency and productivity as well as addressing current challenges in agriculture. In this section, examples of studies demonstrating the positive effects of natural biostimulants on agricultural productivity are presented, highlighting the results obtained in different crops and the NBs involved (Table 8).

Table 8. Effect of NBs on agricultural productivity.

Crop	NB Type	Effect of Productivity on Crops	Scale	Refs.
Corn	Seaweed Extract	Increases grain yield, crop residue, and improves nutritional quality	Field	[149–151]
Grapes	Seaweed Extract	Increases grape production, improves stress resistance, and increases polyphenol content	Greenhouse	[152–154]
Tomato	Seaweed Extract	Increases fruit yield and quality	Greenhouse	[155–157]
Lettuce	Seaweed Extract	Higher yield increase and increases shoot growth	Greenhouse	[158–160]
Strawberries	Seaweed Extract	Improves fruit quality and flavor, higher yield	Greenhouse	[132,161]
Onion	Seaweed Extract	Increases bulb diameter and weight	Field	[162,163]
Potato	Seaweed Extract	Increases tuber yield and quality	Field	[164,165]
Corn	IAA	Stimulates vegetative growth and increases grain production	Greenhouse	[166–168]
Lettuce	IAA	Increases biomass	Greenhouse	[169]
Potato	IAA	Promotes tuber growth and improves yield	Greenhouse	[170–172]
Onion	IAA	Increases bulb size and enhances production	Greenhouse Laboratory	[173–175]
Quinoa	IAA	Boosts grain yield and improves quality	Field	[176,177]

Table 8. *Cont.*

Crop	NB Type	Effect of Productivity on Crops	Scale	Refs.
Wheat	IAA	Stimulates plant growth and increases yield	Field	[178,179]
Tomato	IAA	Improves rooting, increases fruit production, and enhances antioxidant content	Greenhouse	[180,181]
Soybean	IAA	Improves root development and increases production	Greenhouse	[182,183]
Rice	IAA	Promotes rooting and improves yield	Field	[184,185]
Broad Beans	IAA	Stimulates vegetative growth and increases production	Greenhouse	[183,186]
Grapes	IAA	Enhances root formation and increases yield	Greenhouse	[187–189]
Corn	Cytokinins	Stimulates cell division and increases yield	Greenhouse	[190,191]
Rice	Cytokinins	Promotes grain growth and improves yield	Greenhouse	[192,193]
Wheat	Cytokinins	Increases the number of grains per spike and improves production	Field	[194–196]
Soybean	Cytokinins	Improves vegetative growth and increases production	Greenhouse	[197,198]
Tomato	Cytokinins	Stimulates flower formation and increases yield	Greenhouse	[28,199]
Potato	Cytokinins	Promotes tuber development and improves yield	Field	[200,201]
Grapes	Cytokinins	Enhances cluster size and quality	Greenhouse	[202,203]
Strawberry	Cytokinins	Increases stolon formation and improves production	Greenhouse	[204,205]
Strawberry	Cytokinins	Stimulates bud break and improves yield	Greenhouse	[206]
Citrus	Cytokinins	Increases fruit size and improves production	Greenhouse	[207,208]
Onion	Humic Acids	Enhances bulb yield, improves quality and disease resistance	Greenhouse	[209,210]
Corn	Humic Acids	Improves nutrient absorption and increases yield	Greenhouse	[28,211]
Wheat	Humic Acids	Increases grain size and weight	Greenhouse	[212,213]
Rice	Humic Acids	Boosts the number of spikes and improves production	Greenhouse	[214,215]
Tomato	Humic Acids	Enhances fruit quality and increases yield	Greenhouse	[216,217]
Beans	Humic Acids	Improves vegetative growth and increases production	Field	[218]
Onion	Humic Acids	Increases bulb size and quality	Greenhouse	[219,220]
Carrot	Humic Acids	Promotes root development and improves production	Greenhouse	[221]
Lettuce	Humic Acids	Stimulates leaf growth and increases yield	Greenhouse	[222]

4.4. Limitations and Challenges of NBs by SSF

Despite the benefits of NBs in sustainable agriculture, some limitations and challenges need to be considered. These aspects can affect their practical application and widespread adoption in agricultural production. Some of the main limitations and challenges of this study are as follows.

4.4.1. Standardization Issues in NB Production by SSF

In this section, we address some standardization issues that may arise in the process of NB production by SSF. Although SSF offers advantages in terms of cost, efficiency, and small-scale production, there are challenges that need to be addressed to achieve standardized and consistent production of high-quality biostimulants [223]. The following are some common limitations.

Substrate variability: the choice of substrate used in SSF can vary depending on the type of microorganism and production objective. However, the chemical composition and physical properties of substrates can vary, which could affect the quality of NBs.

Control of SSF conditions: SSF conditions, such as temperature, humidity, pH, and substrate/microorganism ratio, are crucial for the growth and activity of microorganisms. Without proper control of these conditions, there may be variations in the production of bioactive metabolites and enzymes [79], which can affect the quality and efficacy of NBs.

Scalability of production: the large-scale production of NBs by SSF can be challenging because of the need to maintain optimal fermentation conditions and ensure the quality of the final product. Scalability of production requires optimization of fermentation parameters, selection of suitable equipment, and design of efficient processes that meet quality standards and market demands [47].

Addressing these standardization issues in the production of NBs by SSF will require a combination of scientific research, development of new methodologies, collaboration between academia, industry, and regulatory bodies, and the adoption of good manufacturing practices. These efforts will contribute to ensuring the quality, consistency, and efficacy of NBs produced by SSF, thereby facilitating their reliable and sustainable application in agriculture.

4.4.2. Challenges in the Application of NBs from SSF in Sustainable Agriculture

In this chapter, we explore some difficulties that may arise in the application of NBs produced by SSF in sustainable agriculture. Although NBs offer numerous benefits for improving crop performance and quality, as shown in Table 7, there are still specific challenges related to their application in sustainable agricultural systems. The following are some possible difficulties.

Regulation and Standards: the lack of updated regulations in many countries regarding the use of NBs can hinder their application in sustainable agriculture, as evidenced by a critical analysis [224]. The lack of clear definitions and standards can create uncertainty regarding dosing and the frequency of application, which could hinder their widespread adoption.

Interaction with other inputs: the interaction of NBs with other inputs can be complex and may require adjustments in application practices to avoid possible negative interactions or decrease in product efficacy [225]. In sustainable agriculture, it is common to use multiple inputs such as organic fertilizers, biological pesticides, and beneficial microorganisms.

Adaptability to different crops and agronomic conditions: NBs can have different effects depending on crop type and agronomic conditions [20]. Some NBs may work more effectively on certain crops or at certain phenological stages, requiring a detailed understanding of their mode of action and proper adaptation to the specific conditions of each crop.

Farmer capacity building: the adoption of NBs in sustainable agriculture may require increased awareness and knowledge among farmers [226]. It is important to educate farmers about the benefits and proper use of NBs, as well as providing training and technical assistance to maximize their effectiveness on crops.

Overcoming these difficulties in the application of NBs produced by SSF in sustainable agriculture requires a comprehensive approach involving researchers, farmers, businesses, and the government. It is important to encourage the research and development of best practices, establish clear regulations, and promote training and awareness among key players in the agricultural supply chain.

4.4.3. Factors Limiting the Effectiveness of Natural Biostimulants Produced by SSF in Different Crops

The effectiveness of NBs produced by SSF can be influenced by various factors in different crops. Some of these factors include the genetic variability of crop varieties, environmental conditions, such as temperature and humidity, and nutrient availability in the soil. Additionally, NBs produced by SSF interact with other agricultural inputs, such as fertilizers and pesticides. NBs are not a universal solution and should be combined with good agricultural practices such as crop rotation and proper soil management, which can affect their effectiveness [169,227]. Further research is needed to better understand the response of different crops to NBs produced by SSF and to optimize SSF conditions, valorizing waste to maximize their benefits in sustainable agriculture.

5. Conclusions and Future Research Perspectives

5.1. Conclusions

In this section, we present our conclusions and future research perspectives regarding the production of NBs from SSF. In this review, we have analyzed the use of NBs in agriculture, their production by SSF, and their effects on crops. The main conclusions derived from this study are as follows:

NBs are a promising tool to improve crop development and performance. Their use can contribute to more sustainable agriculture by reducing reliance on synthetic chemicals.

SSF is an efficient technique for producing NBs from organic substrates. This method offers several advantages, such as the valorization of agricultural and agro-industrial waste.

NBs act through various bioactive molecules, such as auxins, cytokinins, alginic acids, humic acids, and other compounds. These molecules can modulate physiological and metabolic processes in plants, improving nutrient uptake, rooting, biotic and abiotic stress tolerance, and crop quality.

However, challenges and limitations still need to be addressed to maximize the effectiveness of NBs. These include standardization of production, optimization of dosages and application, adaptations to different crops and environmental conditions, and understanding interactions with other agricultural inputs.

5.2. Future Research Prospects

The following are future research perspectives. A multidisciplinary approach is required to advance the field of NBs from SSF. Some promising areas of research include the following.

Further studies are needed on the mechanisms of action of NBs at the molecular and cellular levels. This will help to better understand how they interact with plants and modulate specific physiological processes.

Research on the optimization of NB production processes produced by SSF. This involves improving the substrates, selecting efficient microorganisms, and optimizing SSF conditions to obtain high-quality and consistent products.

Investigation of the effectiveness of NBs in different agricultural systems and environmental conditions. This includes field and greenhouse studies that analyze the impact of biostimulants on various crops, regions, and agricultural practices.

Research on the interaction of NBs with other agricultural inputs, such as bio-fertilizers and bio-pesticides is needed to optimize their combined use and minimize potential negative effects.

In conclusion, NBs produced by SSF have significant potential for improving agricultural productivity and promoting sustainable farming practices. However, further research, development, and innovation are needed to overcome these challenges and maximize their efficacy for different crops and environmental conditions. An integrated approach that combines scientific research, collaboration among different stakeholders, and the implementation of science-based agricultural practices is essential to fully harness the benefits of NBs in sustainable agriculture.

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Abbreviations

Natural biostimulants	(NBs)
Solid-state fermentation	(SSF)
The European Biostimulants Industry Council	(EBIC)
Humic substances	(HS)
Hormone-containing products	(HCP)
Amino-acid-containing products	(AACP)
Indole-3-acetic acid	(IAA)
Abscisic acid	(ABA)

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