Received: 30 April 2024 | Accepted: 10 July 2024

DOI: 10.1111/1751-7915.14534

MICROBIAL BIOTECHNOLOGY Open Access

MINI REVIEW

Biopreparations for the decomposition of crop residues

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Funding information
The Agency for Restructuring and Modernisation of Agriculture, Poland, Grant/Award Number: 00077. DDD.6509.000167.2022.05

Abstract

Recently, there has been growing interest in biopreparations that intensify the decomposition of crop residues. These preparations can promote nutrient cycling and soil fertility, ultimately supporting healthy plant growth and increasing agricultural productivity. However, the development and commercialization of biopreparations poses unique challenges. Producers of biopreparations struggle to develop highly effective preparations, which then face regulatory hurdles and must win market acceptance. This literature review provides up-to-date data on microbial preparations available commercially on the European market, along with information on current relevant regulations. Challenges for the development and commercialization of new biopreparations are also discussed. The development and commercialization of biopreparations require a comprehensive approach that addresses the complex interplay of microbial and environmental factors. The need for more specific regulations on biopreparations for decomposing crop residues, clearer instructions on their use, and further research on the overall impact of biopreparations on the soil metabolome and optimal conditions for their application were indicated. Moreover, manufacturers should prioritize the development of high-quality products that meet the needs of farmers and address concerns about environmental impact and public acceptance.

INTRODUCTION

The European Union's strategic European Green Deal (included in the Farm to Fork Strategy) aims to promote sustainable agricultural production to ensure food security and achieve climate neutrality (Havryliuk et al., 2022; Nazranov et al., 2021). This includes a radical reduction in the use of pesticides and the promotion of organic farming practices (European Commission, 2019, 2020a, 2020b). As a result, both scientists and farmers are looking for effective natural preparations that enhance agricultural productivity.

Biopreparations offer one such alternative to chemical plant protection products (Ayilara et al., 2023; Fenibo et al., 2022; Narwade et al., 2023). Biopreparations can be used as biopesticides, biofertilizers, biostimulants, or biodegradation stimulators (Chakraborty et al., 2023; Marwal et al., 2022; Narwade et al., 2023; Parajuli et al., 2022; Pylak et al., 2019; Toader, Chiurciu, Filip, Burnichi, et al., 2020; Upadhyay et al., 2020). Based on their composition, biopreparations used in agriculture can be classified as fungal, bacterial, bacterial/fungal-enzymatic, bacterial-fungal, or enzymatic (Toader, Chiurciu, Maierean, Sevciuc, et al., 2020; Figure 1).

Biofertilizers are one of the best ways to increase or maintain the current rates of food production while ensuring environmental stability. They contain selected microorganisms such as *Azospirillum*, *Azotobacter*, *Rhizobium*, and *Pseudomonas fluorescens* (Divya et al., 2023; Upadhyay et al., 2021; Vishal et al., 2023) and/or various plant extracts (fruit, leaf, microalgal seaweed; Anli et al., 2023; Bairwa et al., 2023). Biofertilizers improve soil quality and support plant growth as a result of the synthesis of growth regulators, biocontrol of phytopathogens, or induction of immunity during stressful conditions. They can also improve the

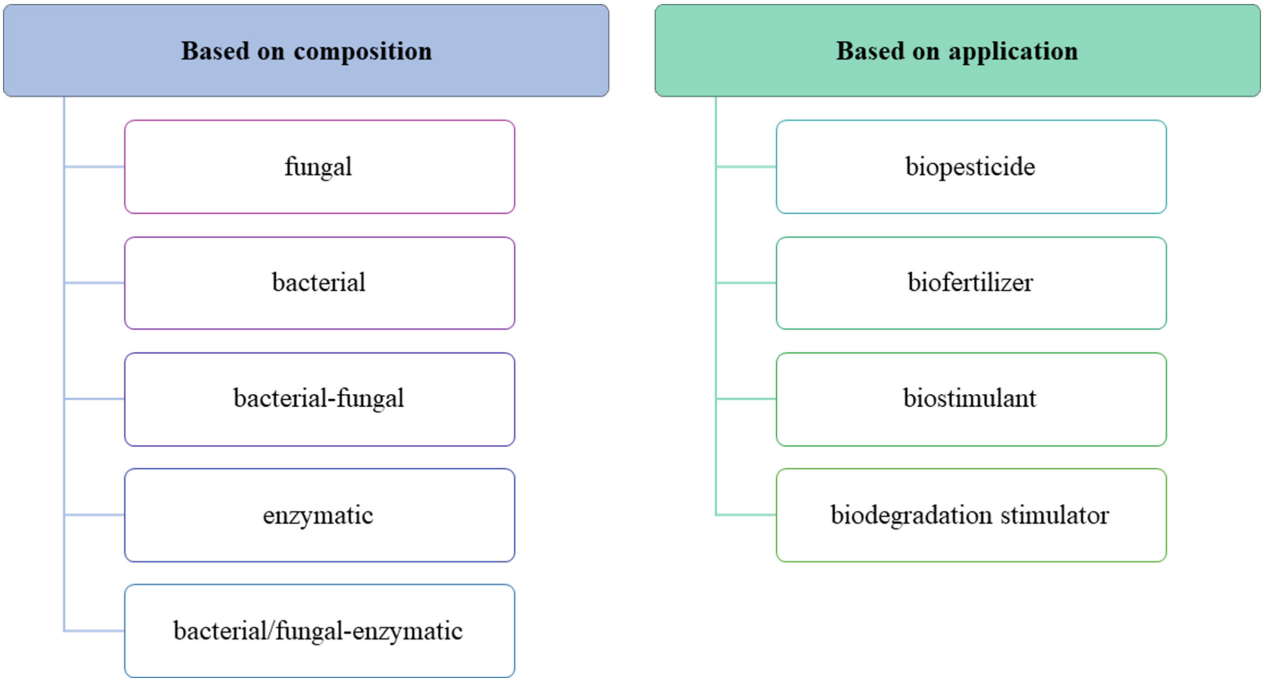

FIGURE 1 Classification of biopreparations.

absorption of hard-to-access elements, due to their ability to solubilize phosphorus, potassium, and zinc (Kumar et al., 2022; Singh et al., 2017). Biopesticides may be based on the action of microorganisms (e.g. bacteria such as *Bacillus thuringiensis* or moulds such as *Verticillium leconi*, *Metarhizium anisopliae*, or *Trichoderma viride*) or plant extracts (e.g. neem oil, citronella oil, or garlic water extract; Chakraborty et al., 2023; Marwal et al., 2022; Narwade et al., 2023; Parajuli et al., 2022; Steglińska et al., 2022; Upadhyay et al., 2020).

The EPA (United States Environmental Protection Agency) lists 390 commercially available biopesticides. Among these, 53 contain bacteria of the genus *Bacillus* as the active substance and 11 use *Pseudomonas* sp. Other microorganisms used in biopesticides include *Agrobacterium*, *Alternaria*, *Aspergillus*, *Aureobasidium*, *Autographa*, *Beauveria*, *Trichoderma*, and *Streptomyces* (Biopesticide Active Ingredients|US EPA). A separate group of biopreparations are accelerators for the decomposition of plant waste in agriculture (Maharjan et al., 2022; Sivaramanan, 2014). Such biopreparations intensify the decomposition of organic residues and the circulation of nutrients in soil (Kotwica et al., 2021).

The growing interest in biopreparations is contributing to the expansion of the market for biotechnological solutions (Havryliuk et al., 2022; Kyrychenko, 2015; Nazranov et al., 2021). The global biotechnology market is projected to reach 2 trillion USD in 2025, with agricultural and environmental biotechnologies constituting 5% of the market. A significant part of agricultural biotechnology is associated with microbial biopreparations (Kyrychenko, 2015). However, the research literature on biopreparations is still insufficient. This applies especially to biopreparations for the decomposition of crop residues.

Despite the growing interest in biopreparations for crop residue decomposition, the current state of this field is characterized by considerable uncertainty. The existing regulatory framework is often ambiguous, resulting in a lack of standardization and consistency in the production and marketing of these products. The increasing recognition among farmers of the importance of efficient residue management in maintaining soil health and optimizing crop yields has led to a growing demand for biopreparations. However, the absence of clear guidelines and regulatory oversight is precipitating confusion among farmers, as well as researchers embarking on studies related to biopreparations. The aim of this review is to present the current state of knowledge on biopreparations for the decomposition of crop residues in agriculture, with particular attention to the challenges and development opportunities in this field. Furthermore, it will summarize key points regarding the legal and industrial aspects of biopreparations for crop residues decomposition. The review is composed of 4 parts: (1) mechanisms of decomposition of crop residues; (2) discussion on microbial preparations commercially available on the European market; (3) characteristic of biopreparations for decomposition of crop residues and optimal practices for their application; and (4) challenges for the biotechnology industry related to the increased demand for biopreparations and possible solutions. The review ends with conclusions and an indication of the most important problems to be solved in future work.

This literature review summarizes the results of 104 scientific articles published in the last 16 years (32 between 2008 and 2018 and 72 between 2019 and 2024), as the starting point for future work. The articles were selected based on quality and relevance from the databases Google Scholar, PubMed, Web of Science, and Research Gate. This review also examines 8 current legal regulations as well as 34 websites related to biopreparations available on the European market.

MECHANISMS OF DECOMPOSITION OF CROP RESIDUES

Harvest residues are parts of plants that remain on the plot after harvest (Pržulj & Tunguz, 2022). The decomposition of crop residues is important for several reasons. Firstly, it plays a crucial role in nutrient cycling and soil fertility, which ultimately supports healthy plant growth and high agricultural productivity (Bunas et al., 2022; Pržulj & Tunguz, 2022). Decomposition processes release nutrients from the residues, making them available for plant uptake and contributing to the overall nutrient content of the soil. Decomposition helps to maintain soil organic matter levels (Pržulj & Tunguz, 2022; Sánchez et al., 2018; Singh & Sharma, 2020). It also affects soil structure, water retention, and the overall microbial community, all of which impact soil health and ecosystem functioning (Ramteke et al., 2018). Overall, understanding and managing the decomposition of crop residues is crucial for sustainable agriculture and maintaining soil quality, especially on organic farms.

Decomposition involves physical, chemical, and biological processes. Three major processes are involved in terrestrial decomposition: leaching, fragmentation, and chemical alteration (Wang & D'Odorico, 2013). Decomposition of plant residues by microorganisms requires two processes: mineralization and humification of carbon compounds (Pržulj & Tunguz, 2022). The processes of humification and mineralization occur simultaneously and are closely related. Humification products are included in the mineralization process, and vice versa. Approximately, 75–80% of the organic matter introduced into the soil annually (organic fertilizers, plant and animal remains) undergoes mineralization processes, and 20–25% is transformed into specific humus substances (humic acids, humins, fulvic acids; Pikuła & Ciotucha, 2022). Microbial mineralization is a process in which microorganisms convert organic matter into water-soluble inorganic forms. Range of products of the mineralization also depends on the conditions of process. Anaerobic conditions result in mainly CO_2, H_2O, H_2S, and CH_4, while aerobic conditions result in CO_2, H_2O, NO_3^-, PO_4^{3-}, SO_4^{2-}, K^+, and Ca^{2+} (Grzyb et al., 2020; Pikuła & Ciotucha, 2022; Pržulj & Tunguz, 2022). Different organic residues show different mineralization patterns, based on their chemical constituents. Plant residues cause rapid immobilization of nitrogen, affecting microbial size and activity, followed by further mineralization. Residues contribute significantly to soil microbial biomass size and activity (Cayuela et al., 2009). Microbial conversion of organic matter is necessary for mineralization of organic nitrogen. One fraction of the converted matter is assimilated by the microbes into their tissue and another part is used for oxidation, to gain energy (dissimilation). The dissimilation-to-assimilation ratio depends on the type of microorganism (Pržulj & Tunguz, 2022). Mineralization rates depend on the type of plant residue. Poorly decomposable types are the main source of particulate organic matter. Highly decomposable types are correlated with microbial biomass. For example, the aboveground mass of meadow grasses and the aboveground mass and roots of clover have high mineralization rates, whereas small tree branches and barley straw have slow mineralization rates (Semenov et al., 2019). Cover crops, which are grown primarily to benefit the soil and the environment in agricultural systems, also play an important role in the decomposition of organic matter and the composition of microorganisms in the soil. The diversity of cover crop residues has been found to enhance microbial decomposition and increase the soil respiration rate (Shu et al., 2021). Similar results have been reported by Nevins et al. (2018), who showed that soil microbial communities were significantly different based on cover crop treatment. The structure of the soil microbiome changed during the decomposition period, and the amount of cellulolytic bacteria (*Agromyces*, *Agrobacterium*, and *Bacillus*) increased (Nevins et al., 2018).

Microbial humification is the process by which organic matter is converted into structurally refractory substances. It is fundamental to the overall humification process. Products of humification include humic acids, humins, and fulvic acids (Bui et al., 2023; Pikuła & Ciotucha, 2022). Microbial humification can be enhanced by the addition of biotic catalysts during composting, which increase the content of humic substances. Laccase has been found to be particularly well-suited for promoting humification, due to its environmentally friendly nature and high efficiency (Bui et al., 2023). Lowering the pH level during the composting process in a reactor can also enhance microbial humification (Zhao et al., 2023). Adding plant residues with different carbon-to-nitrogen (C:N) ratios to the soil can influence both the decomposition process and the composition of the microbial community. Higher C:N ratios have been shown to enhance microbial biomass, although at the expense of reducing the bacteria-to-fungi ratio. The ratio of Gram-positive to Gram-negative bacteria is also reduced significantly (Liang et al., 2017).

The C:N ratio plays a key role in determining whether the decomposition process is accelerated (positive priming effect) or slowed (negative priming effect). For example, organic material such as maize residues with a low C:N ratio can promote a positive priming effect in the short term, while material with a high C:N ratio, such as straw or wood chips, can initially slow down decomposition before ultimately accelerating it. The intensity of the priming effect is also influenced by the rate at which fresh organic matter is input into the system (Liang et al., 2017; Yu et al., 2020). Similarly, it has been reported that microbial biomass and mineralization are significantly affected by the soil type and rate of residue addition, although not by N level (Roberts et al., 2015).

During microbial decomposition, the enzymatic activity of microbial strains and the production of extracellular hydrolases are essential processes. Plant residues contain large amounts of cellulose, hemicellulose, starch, and lignin that need to be broken down (Chertov et al., 2007; Grzyb et al., 2020; Madhavan et al., 2017). Some microorganisms, such as *Bacillus* sp. and *Penicillium* sp., have a wide range of activity and are capable of degrading all of these substances. Bacteria and fungi are the primary decomposers in soil ecosystems, with bacteria being responsible for the initial breakdown of organic matter and fungi playing a key role in the later stages of decomposition (Hellequin et al., 2018; Xu et al., 2020). Table 1 lists microorganisms participating in the degradation of crop residues and their enzymatic abilities.

BIOPREPARATIONS FOR DECOMPOSITION OF CROP RESIDUES

The use of microorganisms in conjunction with fertilization using straw has been shown to effectively enhance winter wheat yield, particularly in challenging environmental conditions (Kotwica et al., 2014). The addition of shredded straw along with microorganisms has been shown to increase the total number of microorganisms in the soil. This increase in the number of active cells can positively influence the rate of decomposition of organic matter and the cycling of nutrients (Kotwica et al., 2021). The use of microbiological preparations for decomposing crop residues can have a positive effect when high doses of straw are incorporated into cereal crop rotations, when straw is used under cereal cultivation, and in no-till agriculture, especially when environmental conditions are unfavourable for natural decomposition of crop residues (Rusakova, 2016).

Table 2 presents the compositions of several commercially available preparations for the degradation of plant residues that have been characterized in the literature. A field study conducted to assess the effectiveness of BioSistem POWER SC showed that the biopreparation increased the emission of carbon dioxide from the soil, as well as the level of cellulolytic activity (by 23–34% depending on the rate of use) and the antifungal activity of the soil (by 2.5–3.0 times compared to the control group). However, the active bacterial strains and moulds present in the preparation contributed to inhibit fungal pathogens in the soil (Bunas et al., 2022). Similar results were observed during the treatment of crop residues (spring barley and pea) with Stubble Biodestructor. This research was performed in an experimental field over a five-year period (Panfilova, 2021). The biopreparation increased the number of cellulolytic microorganisms in the soil by 2.8×10^6 up to 3.6×10^6 CFU/g and also increased the total number of bacteria. Treatment of crop residues with water (control trial) resulted in a smaller increase in the total number of bacteria, from 6×10^5

TABLE 1 Microorganisms involved in the biodegradation of plant residues.

Enzymatic abilities	Bacteria	Fungi	Literature
Cellulolytic	*Cellulomonas* sp., *Cellvibrio* sp., *Cytophaga* sp., *Bacillus* sp., *Clostridium* sp., *Paenibacillus* sp.	*Trichoderma* sp., *Fusarium* sp., *Mycogone* sp., *Penicillium* sp., *Aspergillus* sp.	Eida et al. (2011); Fathallh Eida et al. (2012); Grzyb et al. (2020); Hema et al. (2023); Madhavan et al. (2017)
Hemicellulolytic	*Bacillus* sp., *Streptomyces* sp., *Actinomycetes* sp.	*Aspergillus* sp., *Mucor* sp., *Rhizopus* sp., *Botrytis* sp., *Cladysporium* sp., *Trichosporon* sp., *Cryptococcus* sp., *Aureobasidium* sp., *Penicillium* sp.	Eida et al. (2011); Grzyb et al. (2020); Robl and Mergel (2019)
Amylolytic	*Pseudomonas* sp., *Amylobacter* sp., *Bacillus* sp., *Streptomyces* sp., *Clostridium* sp.	*Aspergillus fumigatus*, *Penicillium citrinum*	Benjamin et al. (2013); Grzyb et al. (2020); Ramzan et al. (2016)
Pectinolytic	*Bacillus* sp., *Pseudomonas* sp., *Flavobacterium* sp., *Propionibacterium* sp.	*Penicillum* sp., *Trichoderma reesei*, *Fusarium oxysporum*, *Aspergillus fumigatus*	Benjamin et al. (2013); Grzyb et al. (2020); Okonji et al. (2019)
Ligninolytic	*Azotobacter* sp., *Xanthomonas* sp., *Pseudomonas* sp., *Agrobacterium* sp., *Actinobacteria* sp., *Bacillus* sp.	*Ganoderma lucidum*, *Irpex lacteus*, *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp., *Chaetomium* sp.	Grzyb et al. (2020); Rehman et al. (2022); Singh and Upadhyay (2019); Xu et al. (2020); Yang et al. (2021)

TABLE 2 Biopreparations for crop residue decomposition described in the literature.

Product	Producer	Composition	Form	Literature
BioSistem POWER SC	Institute of Agroecology and Environmental Management of NAAS Kyiv, street Metrolohichna, 12	*Paenibacillus*, *Azotobacter*, *Enterobacter* and micromycetes of the genus *Trichoderma*	Suspended concentrate	Bunas et al. (2022)
Stubble Biodestructor	BTU-Center Office 1, 1/34, Akademika Amosova st. Sofiyivska Borshahivka, Kyiv oblast 08138, Ukraine	Bacteria-antagonists of pathogenic fungi to plants and bacteria, soil bacteria phosphate-mobilizers, natural endophytic and soil bacteria nitrogen-fixing bacteria, lactic acid bacteria (LAB), producers of pulp, biofungicides, phytohormones, vitamins, amino acids, macro- and microelements	Liquid	Panfilova (2021)
Bactofil Cell	Nutri-Tech Solutions Pty Ltd (NTS) 7 Harvest Road, Yandina, Queensland, Australia	*Cellvibrio* sp., *Pseudomonas fluorescens* microorganism variants, macro- and microelements, enzymes, and other soil-conditioning ingredients; content of no less than 3×10^9 cells/mL	Liquid	Milev et al. (2015)
Nutri Life Accelerate	Nutri-Tech Solutions Pty Ltd (NTS) 7 Harvest Road, Yandina, Queensland, Australia	*Trichoderma lignorum* and *resei*, *Aspergillus* sp., *Penicillium* sp., *Chaetomium globosum*, *Paecillumyces* sp., *Phanerochaete chrysosporium*, *Paenibacillus polymyxa* *Streptomyces* sp.	Liquid	Milev et al. (2015)
Amalgerol premium	Nutri-Tech Solutions Pty Ltd (NTS) 7 Harvest Road, Yandina, Queensland, Australia	Extract from sea weeds and mineral oils	Liquid	Milev et al. (2015)
EM 'Naturally Active' (EM Naturalnie Aktywny)	Greenland Technologia EM sp. Z o.o. Trzcianki 6, 24-123 Janowiec n/Wisłą, Poland	Photosynthetic bacteria, LAB, yeast, actinobacteria, *Azotobacter* sp. bacteria, and cane molasses	Liquid	Bauza-Kaszewska et al. (2022)
DBC Plus type L	BioArcus Sp. z o. o. Białostocka 22/9, 03-741 Warszawa, Poland	Air-dried and lyophilised bacteria of the genus: *Bacillus* sp., *Artherobacter* sp., *Acinetobacter* sp., *Pseudomonas* sp., *Enterobacter* sp., and surfactants, buffers, and enzymes that significantly accelerate the removal of fatty deposits	Powder	Worwąg et al. (2020)
Radivit	Neudorff Blankschmiede 6, 31855 Aerzen, Germany	Live microorganisms (bacteria and fungi)	Not mentioned	Worwąg et al. (2020)
DBC Plus R5	BioArcus Sp. z o. o. Białostocka 22/9, 03-741 Warszawa, Poland	Various strains of microorganisms	Powder	Worwąg et al. (2020)

up to 15.3 × 10^5. The most effective decomposition occurred after treatment with Stubble Biodestructor with the addition of ammonium nitrate. Avdeeva et al. (2016) have also reported that the addition of ammonium nitrate can accelerate the decomposition of crop residues, providing the best source of nitrogen nutrition for the manifestation of hydrolytic activity by the strains *Bacillus subtilis* IMB B-7516 and *Bacillus licheniformis* IMB B-7515 (Avdeeva et al., 2016). However, Rinkes et al. (2016) found that increased nitrogen availability suppresses the lignin-degrading activities of enzymes (Rinkes et al., 2016).

Researchers have also compared the effectiveness of different commercial biopreparations. Milev et al. (2015) conducted a four-year comparative study of Bactofil Cell, Nutri Life Accelerate, and Amalgerol premium. These biopreparations are utilized for various agricultural applications, including the decomposition of cellulose and wheat cultivar Enola residues. Bactofil Cell is a bacterial-fungal-enzymatic biopreparation, Nutri Life Accelerate is a bacterial-fungal, and Amalgerol premium is an enzymatic biopreparation. Bactofil Cell and Nutri Life Accelerate were found to have a positive effect on the decomposition process and grain yield, which confirms that biopreparations can be a good addition to agriculture methods (Milev et al., 2015). In a three-year study on a field of winter wheat, the microbial inoculant EM Naturally Active was found to increase total organic carbon and the total nitrogen concentration in the soil, as well as the number of bacteria and total number of microorganisms (Bauza-Kaszewska et al., 2022).

There is lack of data on the influence of biopreparations on the soil metabolome. Studies have focused mainly on the impact of biopreparations on selected parts of the microbial community that are necessary for decomposition. There is also a lack of data on the effects of chemical fertilizer as along with biopreparations. Most studies are conducted under controlled conditions with limited field tests, which is insufficient. Future work should compare the effectiveness of biopreparations in field studies under diverse conditions, encompassing various agricultural methods and soil properties.

Commercially available biopreparations can vary depending on the region of sale, because of national and international regulations, and also depending on the needs of farmers for cultivating certain types of crops. Table 3 summarizes information about biopreparations available on the European market that can be found on the official producers' websites. Biopreparations are available on the European market for the decomposition of straw (BACTIM® SŁOMA; Bi Słoma; SŁOMER), as well as for the green parts of crop residues, leaves, and fruits (Bactorol Plus; Bi Compost; ProBios Plus Komposter; RewitalPRO+). Biopreparations are also available for the decomposition of crop residues, liquid manure, and stable manure (KOMPOSTIL). Products in liquid form are more common than products in loose form. This may be due to their simple method of production, which does not require additional steps (e.g. lyophilization), and therefore lower price. Loose form products are often referred to simply as 'powder', without specifying whether they are in lyophilized or dried form (Bi Compost; Bi Słoma).

Effectiveness of commercially available biopreparations is often not supported by any scientific research, and their composition is not controlled, and there is no guarantee that it is entirely consistent with the declarations of the manufacturers. The full compositions of biopreparations are often treated as trade secrets by manufacturers. Producers usually do not specify species, but only the genus or group of bacteria (e.g. *Bacillus* sp., LAB). The amounts of microorganisms may also be unspecified. Such information is important for scientists to evaluate and compare biopreparations for customers. There are no universal guidelines for what information should be included in instruction manuals for farmers. Instructions provided by producers of biopreparations can vary widely, ranging from vague advice such as avoiding use during drought or strong solar radiation to specific details on the optimal soil pH or humidity for maximizing the effectiveness of the biopreparations. To ensure efficient and effective use, instruction manuals should provide information on optimal and critical conditions for application, including details on rates of application depending on the type of agricultural crop, compatibility with other agrochemicals like herbicides and chemical fertilizers, potential mixing with liquid manure, recommended agrotechnical treatments post-application (such as disking or tillage), and the necessity of using unchlorinated water for dilution. Providing this level of detail ensures efficient and effective use of the biopreparation while minimizing potential risks or adverse effects.

Since November 2022, the EU has implemented four regulations intended to accelerate the approval of microorganisms for use as active substances in plant protection products, and to increase the pool of such preparations on the market (Commission Regulation (EU) 2022/1438, 2022; Commission Regulation (EU) 2022/1439, 2022; Commission Regulation (EU) 2022/1440, 2022; Commission Regulation (EU) 2022/1441, 2022). Details of these regulations are given in Table 4.

Under the current EU regulations, all biopreparations are registered as either biopesticides or biostimulators, without specific differentiation of other types of biopreparations. As a result, biopreparations for post-harvest residues decomposition, for example, are registered as microbial biostimulators. Moreover, the registration procedure for biostimulants is cheaper and less complicated than for biopesticides, which

TABLE 3 Biopreparations for decomposition of crop residues available on the European market[a].

Product	Producer	Composition	Form	Website
SŁOMER	AGROBIOS Lutowa 5, 64-300 Nowy Tomyśl, Poland	*Bacillus* sp., lactic acid bacteria (LAB), ecological sugar cane molasses, revitalized water	Liquid	(SŁOMER)
KOMPOSTIL		LAB, *Bacillus* sp., yeasts, phototrophic bacteria, ecological sugar cane molasses, revitalized water	Liquid	(KOMPOSTIL)
BactoRol Plus	Bacto-Tech sp.z o.o. Polna 148, 87-100 Toruń, Poland	Five species of *Bacillus* sp.	Liquid	(Bactorol Plus)
RewitalPRO+	BIO-GEN Pojezierska 97 K, 91-341 Łódź, Poland	Several genera of bacteria (≥1×10^8 CFU/g)	Liquid	(RewitalPRO+)
ProBios Plus komposter	Eko-Natural Przemysłowa 7 L, 11-600 Węgorzewo, Poland	LAB	Liquid	(ProBios Plus Komposter)
BACTIM® SŁOMA	INTERMAG sp. z o.o. Al. 1000-lecia 15G, 32-300 Olkusz, Poland	*Bacillus* sp. (≥5×10^8 CFU/mL), zinc (Zn) – min. 0.07%, iron (Fe) – min. 0.07% (m/m)	Liquid	(BACTIM® SŁOMA)
Bi słoma	ORGANIKA-AGRARIUS Sp. z o. o. Prałkowce 177/1, 37-700 Przemyśl, Poland	*Bacillus* sp. (≥5×10^8–1×10^9 CFU/g) non-pathogenic soil fungi (≥5×10^8 spores/g)	Loose powder	(Bi Słoma)
Bi compost		*Bacillus* sp. (≥5×10^8–1×10^9 CFU/g)	Loose powder	(Bi Compost)
GLEBOSTAN	JMS - GLOBAL Sławomir Hruszka uL. Górna 3, 98-100 Łask	*Bacillus* the total number of viable cells ≥1×10^9 CFU/mL	Powder	(GLEBOSTAN 100 g - 1 Ha - Premiumbakt Preparaty Mikrobiologiczne.)
PK Booster Compost Tea	BIOTABS Biohorti SLU, Carrer Sud 1, 08329 Teia, Spain	Concentrated organic compost, Bat Guano, Kelp, P-releasing bacteria, K-mobilizing bacteria, and selected special ingredients	Suspended concentrate	(PK Booster Compost Tea)
Compost Activator	Microbz Unit 5, Broad Lane Farm, Melksham, SN12 6RJ, United Kingdom	Purified water, beneficial microbes (LAB, yeasts, and hydrocarbon processing bacteria), organic molasses and water	Liquid	(Compost Activator)
Ecostern	SB Soil Plant Kadijkweg 1, 1619PJ Andijk, Netherlands	Bacteria *Bacillus subtilis*, *Azotobacter*, *Enterobacter*, *Enterococcus* and fungi *Trichoderma lignorum*, *Trichoderma viride*, the total number of viable cells 2.5×10^9 CFU/mL	Liquid	(Ecostern - SB Soliplant BV.)
Bactim® Soil	Intrachem Bio Deutschland GmbH & Co. KG Bahnhofstraße 52, 65520 BAD Camberg, Germany	*Bacillus licheniformis* strain B00106: 2.5×10^8 CFU/mL in the form of endospores, *Bacillus subtilis* strain B00105: 2.5×10^8 CFU/mL in the form of endospores, 1.5% N as carbamide	Liquid	(Bactim® Soil.)

(Continues)

TABLE 3 (Continued)

Product	Producer	Composition	Form	Website
RUINEX	Bioenergy LT Staniūnų g. 83, Panevėžys 36,151, Lithuania	*Bacillus mojavensis* MVY-007, *Bacillus amyloliquefaciens* MVY-008, *Bacillus megaterium* MVY-001, *Thrichoderma harzianum* MVY-021 (in total >1.2×10^{12} CFU/L)	Liquid	(Ruinex; Safety Data Sheet - RUINEX.)
NovaFerm® Multi	AGROsolution GmbH & CO. KG Prinz-Eugen-Straße 23, 4020 Linz, Austria	'New cultivar of strains of bacteria, which are UV-resistant and resistant to light, cold and heat. New cultivar of strains of bacteria, which are UV-resistant and resistant to light, cold and heat'	Liquid	(NovaFerm® Multi – AGROsolution; NovaFerm R Multi)
BIONORMA DESTRUCTOR	BioNorma Kyiv, Vaclav Havel Boulevard 4, Ukraine	*Trichoderma harzianum*, *Trichoderma lignorum*, *Pseudomonas fluorescens*, *Pseudomonas aureofaciens*, *Paenibacillus polymyxa*, 1×10^{9} CFU/g	Granules	(Біонорма Деструктор)
Humus Active Grains	Green Ground City of Sofia, Studentski Grad, 2 "Alexander Fall" St, Bulgaria	Organic acids, enzyme substances, watersave complex (a complex of argillaceous minerals), cellulose-decomposing bacteria *Bacillus megaterium*, *Streptomyces abus* and others, Gram-positive bacteria of the *Bacteriaceae* family, nitrogen-fixing bacteria *Azotobacter* sp., *Nitrosomonas* sp., nutrient elements: N, P, K, Ca, Mg, C, Cu, Fe, Mn, Zn	liquid	(Humus Active Grains)
Baikal EM1	Valbrenta Chemicals Ltd ('ВАЛБРЕНТА КЕМИКАЛС' ООД), Belarus; official distributor in Bulgaria: HyperGroup Ltd.	*Lactobacillus brevis* 1×10^{8} CFU/mL, *Lactobacillus rhamnosus* 1×10^{7} CFU/mL, *Lactobacillus buchneri* 1×10^{7} CFU/mL, *Bacillus licheniformis* 1×10^{3} CFU/mL, *Pichia* sp. 1×10^{3} CFU/mL	Liquid	(Baikal EM1; Какво е Байкал EM1?)
AZORHIZ soil bacterial fertilizer	Pannon Trade 9026 Győr, Mayer Lajos 69, Hungary	*Azotobacter chroococcum*, *Azospirillum brasilense*, *Bacillus megaterium*, species-specific rhizobium bacteria	Liquid	(AZORHIZ)

[a]Data for 7 March 2024.

TABLE 4 European Union (EU) regulations for registration of microbial biopreparations.

Legislative body	Date	Regulation
The European Commission	31 August 2022	Commission Regulation (EU) 2022/1438 amending Annex II to Regulation (EC) No 1107/2009 as regards specific criteria for the approval of microbial active substances
The European Commission	31 August 2022	Commission Regulation (EU) 2022/1439 amending Regulation (EU) No 283/2013 as regards the information to be submitted in respect of active substances and specific data requirements for microorganisms
The European Commission	31 August 2022	Commission Regulation (EU) 2022/1440 amending Regulation (EU) No 284/2013 as regards the information to be submitted for plant protection products and the specific data requirements for plant protection products containing microorganisms
The European Commission	31 August 2022	Commission Regulation (EU) 2022/1441 amending Regulation (EU) No 546/2011 as regards detailed uniform principles for the assessment and authorization of plant protection products containing microorganisms

facilitates the introduction of these products on the market.

Table 4 outlines the EU guidelines for biopreparation registration. However, it is essential to note that individual Member States may have supplementary regulations and procedures governing the registration of biopreparations. EU regulations are the most universal regulations that apply to the whole EU territory. The EU's regulatory framework serves as a catalyst for changes in national legislation. The additional regulations are exemplified in this article on British and Polish law.

In the UK, the registration of biopreparations is regulated by the Chemicals Regulation (CR) and the Plant Protection Products (PPPs) regulations. Biopreparations are considered as plant protection product under the UK's Plant Protection Products (PPPs) Regulations. This means that they are subject to the same registration requirements as chemical-based pesticides (Official Controls (Plant Protection Products) Regulations 2020 and Come into Force on 22nd June 2020; Plant Protection Products (Miscellaneous Amendments) (EU Exit) Regulations 2019, and Come into Force on Exit Day) The UK's Health and Safety Executive (HSE) is responsible for enforcing the registration requirements for biopreparations and approving active substances. To register a biopreparation, the manufacturer must submit a dossier to the HSE, which includes information on the product's composition, labelling, and safety data. The HSE requires biopreparation manufacturers to submit data on the product's efficacy, safety, and environmental impact. This includes data on the product's effects on humans, animals, and the environment. Biopreparations can be marketed in the UK once they have been authorized by the HSE. HSE also provides instruction on the labelling, including what must be on the label (composition, use instructions, and safety precautions; HSE – Active Substance Approval; HSE – Biostimulants; HSE – Classification and Labelling; HSE – Products Authorization). It's worth noting that the UK's regulatory framework is subject to change due to Brexit and ongoing developments in EU-UK relations.

In Poland, producers for a long time required only the National Institute of Hygiene in Poland (PZH) certificate. Later, Regulation PE 2019/1009 entered into force, which introduced the definitions of a microbial biostimulator and a non-microbial biostimulator (Regulation (EU) 2019/1009, 2019). At the end of 2023, the legislator finally regulated the market for microbiological products in Poland, introducing definitions of microbiological fertilizing products and agents supporting plant cultivation into the Act on fertilizers and fertilization. According to the definition on the website of the Institute of Soil Science and Plant Cultivation (in Poland), microbiological fertilizer products are defined as containing various components such as microorganisms (including dead or inactive ones), microbial consortiums, substances serving as a medium for these microorganisms and their metabolites, or harmless residual substances from nutrients. These products aim to improve the efficiency of nutrient utilization by plants or fungi, enhance their resistance to abiotic stress, improve quality characteristics, or facilitate the absorption of nutrients from inaccessible forms in the soil (Institute of Soil Science and Plant Cultivation).

Pursuant to the Regulation of the Minister of Agriculture and Rural Development of 1 December 2022, the Institute of Soil Science and Plant Cultivation–State Research Institute in Poland (IUNG-PIB) is authorized to maintain lists of microbiological fertilizer products (Institute of Soil Science and Plant Cultivation; Regulation of the Minister of Agriculture and Rural Development of 1 December, 2022). The current list includes biopreparations for decomposition of post-harvest residues, *Rhizobium* vaccines, and biopreparations to increase the uptake of nitrogen and/or phosphorus. The list includes the date of notification, the trade name of the product, and the name of the reporting company. The composition of the preparation and the scope of use are also provided. As of June 2023, there were 134 products on the list, whereas by March 2024 there were 219 products on the list. This shows that the market for biopreparations is changing very dynamically. A microbiological fertilizer products are kept on the list for a period of two years. After this time, the reporting entity is obliged to submit a written declaration of continued production of the biopreparation with the same quality and composition. Failure to declare within the deadline will result in the product being removed from the list of microbiological fertilizing products.

It is worth noting that microbiological fertilizer products included on the list of natural products that can be used in organic farming are subject to a separate verification and assessment procedures. Not all products included on the IUNG-PIB list can be used in organic farming. It should also be emphasized that IUNG-PIB does not test the effectiveness of the products submitted for inclusion on the list. Their effectiveness is verified only on the basis of their conformity to currently applicable regulations. As mentioned above, microbiological fertilizer products are not subject to strict registration. Therefore, a farmer looking for information about a given product and its effectiveness must rely on information provided by the manufacturer or seek guidance from other sources such as agricultural experts, research studies, or other farmers' experiences.

The National Research Institute (PZH) (Poland) specifies documentation requirements necessary for the conformity certification of biopreparations and biocidal products. In the case of biopreparations, the following information is required:

1. The qualitative and quantitative chemical composition of the products (full chemical name of the substance);
2. The composition of microorganisms (types of microorganisms, preferably species names);
3. The method of application of the biopreparation (technical sheet, instructions);
4. Label design;
5. REACH Card/Product Technical Sheet/SDS/MSDS (if available);
6. A declaration from the manufacturer that the preparation does not contain genetically modified microorganisms;
7. A declaration from the manufacturer that the preparation does not contain pathogenic microorganisms according to Directive 2000/54/Ec of The European Parliament and of The European Council of 18 September 2000 on the protection of workers from the risks associated with exposure to biological agents in the workplace and Regulation of the Minister of Health of 22 April 2005 on harmful factors biological products for health in the working environment and the protection of the health of workers professionally exposed to those factors OJ L 2005 No. 81, item 716, as amended;
8. Tests from an independent laboratory for the detection of the presence of bacteria *Escherichia coli*, *Salmonella* sp., enterococci, *Pseudomonas aeruginosa*, in a 10 mL sample of biopreparation or a mass of 10 g. The range of parameters to be determined may change. Research biopreparations should be made in a laboratory management system. All markings should be made according to appropriate (if possible standardized) test methods. (Documentation Requirements Necessary for the Attestation Process for Biopreparations and Biocidal Products – National Research Institute (PZH) (Poland))

These requirements are aimed at ensuring both the safety of employees during the production of the biopreparations and the safety of consumers. However, they do not require confirmation of the effectiveness of the biopreparations.

CHALLENGES FOR THE BIOTECHNOLOGY INDUSTRY RELATED TO THE INCREASED DEMAND FOR BIOPREPARATIONS AND POSSIBLE SOLUTIONS

Biopreparations are generally considered to be both economical and environmentally friendly alternatives to traditional chemical inputs in agriculture (Choudhury, 2015). Their use is encouraged as part of policies and initiatives to promote green growth (Rodrik, 2014). Consequently, biotechnological practices are increasingly being employed to develop a wide range of products, including biopesticides, biofertilizers, and solutions for waste management, biotransformation, bioremediation, and biodegradation. However, the development and commercialization of biotechnology products still face various challenges (Figure 2). These challenges include securing initial funding and continuing investment, the lengthy and complex process of product development, ethical and societal considerations, regulatory hurdles, intellectual property protection, and building market acceptance. This journey is lengthy and complex and requires significant investments of time, effort, and capital (Saxena, 2020). The biotechnology industry also faces unique challenges in each region where it operates. Central and Eastern European countries entered the biotechnology industry relatively late and faced challenges including a lack of partners in the pharmaceutical industry and limited government support (Rudź, 2020; Szczygielski et al., 2022). In contrast, the United States has a favourable tax

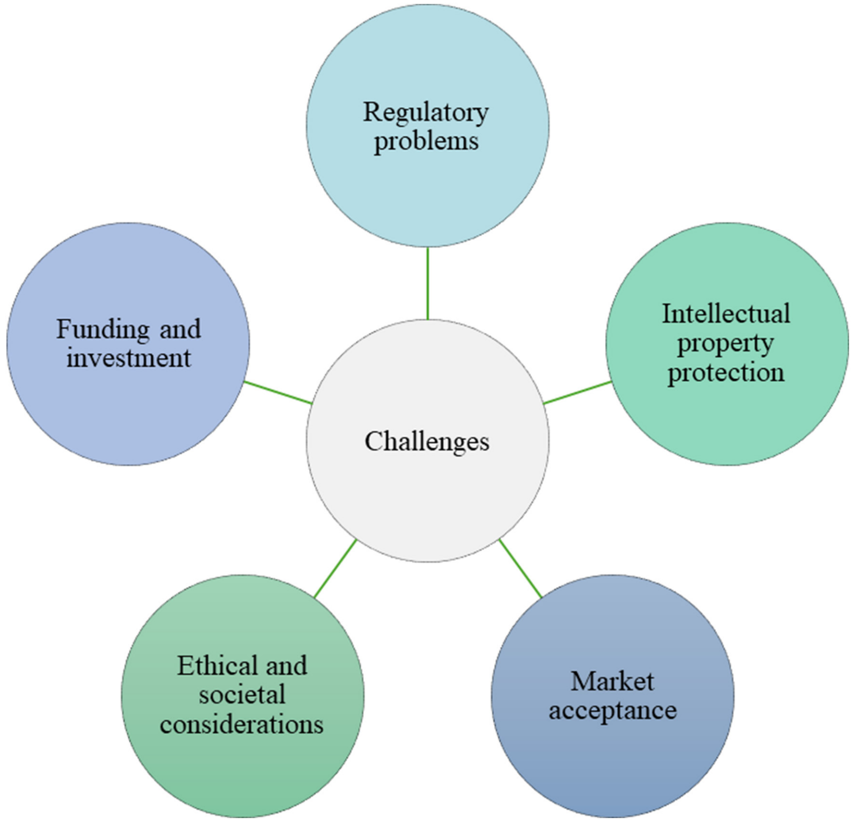

FIGURE 2 Challenges of commercialization in biotechnology.

environment for capital formation and financing small firms, which can facilitate biotechnology commercialization. The Japanese government has made the commercialization of biotechnology a national priority and is financing cooperative inter-industry biotechnology projects. Commercialization of biotechnology in Africa is affected by risk aversion, the complex regulatory environment and export restrictions in African countries, and the availability of natural resources (Elshafei & Mansour, 2018).

The challenges facing the biotechnology industry in general also apply to producers of biopreparations. For example, *Bacillus velezensis* GB03, marketed as Kodiak®, is an eco-friendly substitute for conventional pesticides and fertilizers. It was originally isolated from wheat roots in 1971 in Australia. However, the U.S. Environmental Protection Agency only endorsed GB03 for commercial use in 1998, almost 30 years after it was first isolated (Jang et al., 2023). A simplified diagram of the process of developing biopreparations is given in Figure 3.

The production of biopreparations based on microorganisms or their metabolic products typically begins with the selection of a suitable bacterial strain. One approach is to acquire microorganisms from collections, which provide access to diverse microbial strains for various applications. These collections store and maintain strains in pure forms, ensuring their authenticity and quality over time. Available strains are well known and characterized (Almagambetov et al., 2022; Atit Kanti et al., 2023; Jaroszewska et al., 2023). Some bacteria, such as *Azospirillum*, *Bacillus*, and *Rhizobium*, are known to be suitable for soil inoculation and can improve crop parameters and agricultural productivity (Stojanović et al., 2019; Toader et al., 2022). Another approach is to isolate strains from the environment and then assess their abilities (Galieva et al., 2022; Maiorov et al., 2023). The compositions of microbial biopreparation must be fully defined, and all microorganisms used should be genetically stable, non-pathogenic, and have GRAS status. The microorganisms should be effective at low concentrations, and easy to mass-produce on inexpensive media (Narayanasamy, 2013; Saxena, 2020).

The enzymatic abilities of strains can be characterized in various ways. Preliminary tests can be conducted on media enriched with a substrate specific for each enzyme of interest. These tests can indicate whether the strains possess the required enzymatic activity and provide an initial assessment of the strength or efficiency of that activity (Maiorov et al., 2023; Woźniak et al., 2023). A more sophisticated method for characterizing the enzymatic abilities of strains is metabolomics, which involves the measurement and analysis of metabolites in biological samples using techniques such as NMR spectroscopy, mass spectrometry, and bioinformatics. Metabolomics enables researchers to study metabolic pathways and interactions within microbial communities, providing insights into the metabolic potential of difficult-to-grow bacteria, including

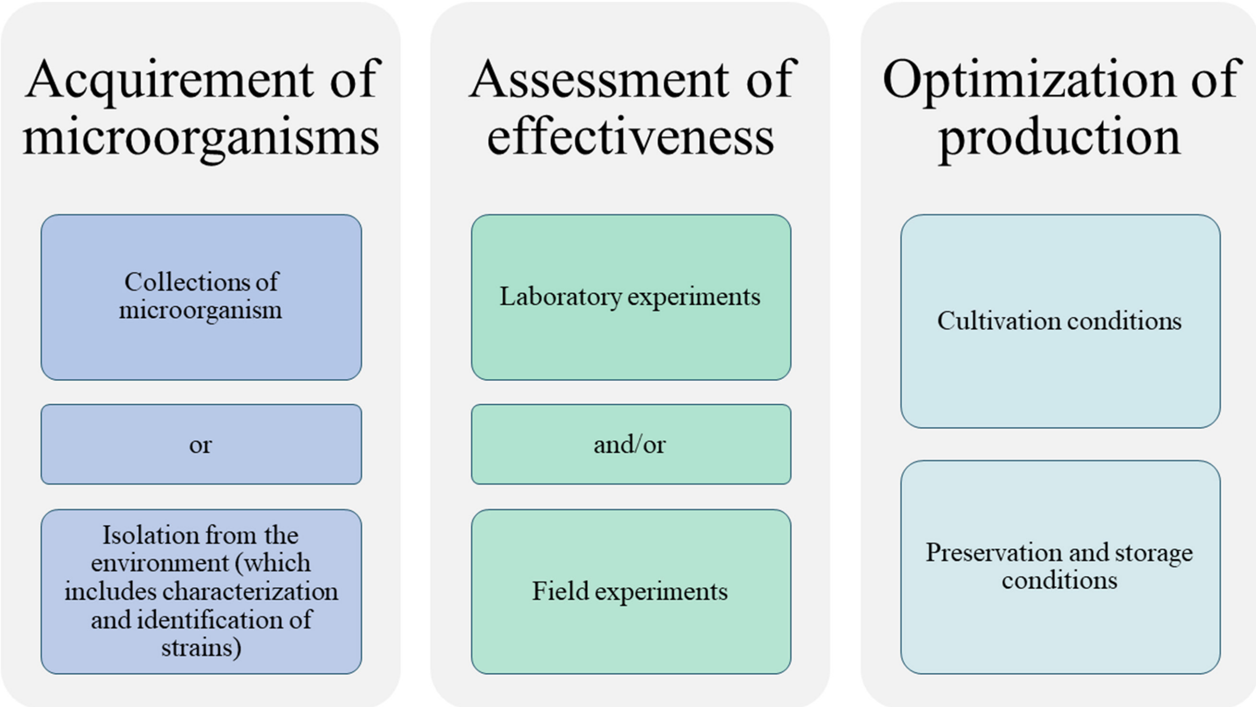

FIGURE 3 Elements during development of microbial biopreparation.

novel bacterial species isolated from environmental samples (Fiorini et al., 2022; Meng et al., 2023; Mohd Kamal et al., 2022; Sieniawska, 2022).

Bacterial strains are commonly identified by genetic methods, based on the 16S rDNA fragment (Pylak et al., 2020; Woźniak et al., 2018). Terletsky et al. (2016) suggested the method of double digestion selective labelling (DDSL) for genetic identification and certification of *Bacillus subtilis* strains showing high resolving power and efficiency. *Bacillus subtilis* strains have similar morphological and cultural features but differ in their metabolite complexes. They are important for the development of microbial biopreparations, including biopreparations for degrading crop residues and promoting plant growth (Terletsky et al., 2016). Lee et al. (2019) proposed a method combining Fourier transform infrared (FT-IR) spectroscopy of bacterial genomic DNA with multivariate analysis for the rapid identification of bacteria at the genus and species levels. This method may be performed in situ, which can be useful because it enables the rapid elimination of pathogenic bacteria during the screening process (Lee et al., 2019).

After selecting the microorganisms, producers must decide whether the biopreparation will be based on a single strain or multiple strains. If multiple strains are used, their antagonistic properties must be investigated, together with their ability to grow in consortia (Galieva et al., 2022; Maiorov et al., 2023). Optimization of the growing medium for bacterial isolates can include optimization of the carbon sources, microelements, pH value, and temperature, as well as shaking (Jiang et al., 2020; Pylak et al., 2021; Stojanović et al., 2019). To assess the effectiveness of biopreparations, it is important to conduct experiments not only in Petri dishes but also under simulated natural conditions (e.g. pot experiments (Shengping et al., 2016)) and in real conditions (e.g. field experiments (Berdnikov et al., 2020; Bunas et al., 2022; Milev et al., 2015; Novokhatsky, 2022; Toader et al., 2019; Toader, Chiurciu, Maierean, Filip, et al., 2020)). The process must then be expanded to the industrial scale. Optimization on an industrial scale usually starts at shake flask level and proceeds to the bioreactor. Shake flasks serve as a conventional tool for initial strain selection and substrate optimization in bioprocessing. The transition to bioreactors is essential in order to fulfil production requirements. However, this transition may present further challenges, particularly with respect to the selection of appropriate stirring and aeration methods. Scaling up can significantly affect the adaptation time and performance of microbial cultures within the bioreactor system (Malkova et al., 2021; Shengping et al., 2016; Stojanović et al., 2019). The final choice of medium and conditions depends on bacterial cultivation results and bacterial profitability. Malkova et al. (2021) report satisfactory results for cultivation of *B. pumilus* on L-broth in a 15L fermenter, achieving up to 10^{10} CFU/ml (Malkova et al., 2021). On the other hand, Shengping et al. (2016) chose a solid-state fermentation using food waste and feldspar, which is positive from both economic and ecological perspectives. It was found that pH, temperature, and humidity had the strongest effects on the number of bacteria. Under optimized conditions, *Bacillus circulans*

Xue-113168 biofertilizer was produced with a spore count of 2×10^9 CFU/g (Shengping et al., 2016).

Preservation and storage can affect the survivability of microorganisms and therefore affect their effectiveness. Shelf life can be extended by using spores as a main microbial agent, adding substances to the product, or applying other preservation methods (Jiang et al., 2020; Malkova et al., 2021; Pylak et al., 2021; Witkowska et al., 2016). Conventional drying, vacuum drying, and lyophilization are commonly used to preserve various strains of bacteria. Preservation methods often include use of a protectant, such as milk or silica gel (Malkova et al., 2021; Pylak et al., 2021; Witkowska et al., 2016). Jiang et al. patented a method of producing microbial fertilizer including synergistic fermentation of *B. megaterium* and *B. subtilis*. The method increases the spore production rate and reduces fermentation time, thereby improving production efficiency. An absorbent prepared from crop stalks and livestock and poultry manure serves as the adsorption matrix for bacterial agents, improving the stability and extending the shelf life of the product (Jiang et al., 2020). *Bacillus* spores have high survivability in soil due to their ability to form endospores, which are metabolically dormant and highly resistant to various environmental stresses (Checinska et al., 2015; Kruglov & Lisina, 2014); therefore, using a spore-based biopreparation can extend the shelf life of the product and improve its effectiveness (Hsieh et al., 2020; Jiang et al., 2020; Kruglov & Lisina, 2014).

CONCLUSIONS

The studies outlined in this review show that microbial and enzymatic biopreparations can accelerate the decomposition of crop residues significantly. However, most studies focus on select components of the microbial community crucial for decomposition processes. There is a lack of research focusing on the overall impact of biopreparations on the soil metabolome. Furthermore, there is limited data regarding the concurrent use of biopreparations and chemical fertilizers. It is imperative to evaluate the efficacy of biopreparations under varied field conditions, agricultural practices, and soil characteristics, in order to better understand their overall effectiveness.

In order to optimize the efficacy of biopreparations and prevent errors during their application, it is essential that the instruction manual includes detailed guidance on optimal and critical parameters. Instruction manuals should contain information on recommended application rates based on specific agricultural crops, guidance on potential compatibility with herbicides, chemical fertilizers, and liquid manure, as well as recommendations regarding post-application agrotechnical treatments, such as disking or tillage. Instruction manuals should also address the necessity of diluting the biopreparations with unchlorinated water. The introduction of universal EU guidelines would be helpful for both customers and manufacturers.

The biopreparations market is also in need of more specific regulation regarding microbiological fertilizer products, which are not subject to stringent registration requirements. The current regulations tend to prioritize formalities, such as administrative requirements and documentation, over substantiation of the effectiveness of biopreparations. Consequently, farmers seeking information about a specific product must primarily depend on details provided by the manufacturer or conduct their own on-farm trials or experiments to assess its effectiveness in their specific conditions.

The commercialization of biopreparations poses unique challenges regarding the development of new products. These include the isolation, characterization, and identification of microorganisms, optimization of the medium, and development of cultivation techniques. Other barriers concern the whole biotechnology industry, such as regulatory hurdles and market acceptance. Manufacturers of biopreparations struggle to develop highly efficient products and to gain acceptance from the market and the general public. Nonetheless, biopreparations offer a great opportunity to maintain current rates of food production while ensuring environmental stability, providing both economical and environmentally friendly alternatives to traditional chemical inputs in agriculture.

AUTHOR CONTRIBUTIONS

Patrycja Rowińska: Conceptualization; writing – original draft; writing – review and editing; visualization; investigation. **Beata Gutarowska:** Writing – review and editing. **Regina Janas:** Writing – original draft. **Justyna Szulc:** Supervision; funding acquisition; writing – review and editing; writing – original draft.

ACKNOWLEDGEMENTS

This work was supported by the Agency for Restructuring and Modernisation of Agriculture, Poland [grant number 00077.DDD.6509.000167.2022.05]. This article was completed while the first author was a Doctoral Candidate at the Interdisciplinary Doctoral School at Lodz University of Technology, Poland.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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How to cite this article: Rowińska, P., Gutarowska, B., Janas, R. & Szulc, J. (2024) Biopreparations for the decomposition of crop residues. *Microbial Biotechnology*, 17, e14534. Available from: https://doi.org/10.1111/1751-7915.14534