

Impact of carrier materials and binding agents on microbial survival and the physical properties of granular biopreparations

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Abstract. The survivability of microorganisms in a biopreparation during storage is important for its successful application. This study evaluated the survivability of a granular biopreparation based on five selected strains of spore-forming bacteria: *Bacillus subtilis*, *Bacillus velezensis*, *Bacillus licheniformis*, *Priestia megaterium*, and *Paenibacillus amylolyticus*. These strains were granulated with different carriers (bentonite, chalk) and binders (water, 50% molasses, post-culture liquid). The results demonstrated high microbial survival, with the chalk-water granulate showing the smallest declines after one year of storage at room temperature: 0.04 log CFU mL g⁻¹ for vegetative cells and 0.03 log CFU mL g⁻¹ for spores. The bentonite-based granules were characterized by high moisture content (up to 65.4%) and lower bulk density. Chalk granules exhibited better compressive strength, exceeding 80 N with molasses as a binder. The tested granule variants also exhibited different dissolution profiles. The chalk-based granules completely disintegrated in water, whereas bentonite-based granules remained intact for 20-28 h, indicating a slower release mechanism. A four-week field study confirmed the prolonged structural integrity of bentonite granules and a corresponding delayed effect on soil microbial populations. The study demonstrates that designing an effective biopreparation requires a holistic approach that simultaneously balances microbial survival, controlled release kinetics, and optimal physical parameters tailored to the agricultural application of the biopreparation granules.

Keywords: biopreparations, granulation, *Bacillus*, spores, storage

1. INTRODUCTION

The advancement of sustainable agricultural practices drives the search for biological alternatives to conventional fertilizers and plant protection products. Microbial preparations play a crucial role in agriculture by enhancing crop yield, improving soil health, and reducing environmental impact. These preparations, containing beneficial microorganisms including bacteria, offer a sustainable alternative to chemical fertilizers and pesticides (Narwade *et al.*, 2023; Pylak *et al.*, 2019; Rowińska *et al.*, 2024). Microbial preparations improve soil fertility by enhancing nutrient cycling and availability. For instance, PGPRs can fix atmospheric nitrogen and solubilize phosphates, making these nutrients more accessible to plants (Sharma *et al.*, 2025). The use of microbial inoculants has been shown to increase crop yields significantly. For example, it leads to higher yields of soybeans, amaranth, and buckwheat (Trufanov *et al.*, 2021). Microbial preparations also serve as biopesticides and bioinsecticides, offering targeted action against pests and pathogens with minimal ecological disruption. This reduces reliance on chemical pesticides and supports sustainable pest management practices (Sharma *et al.*, 2025). The effectiveness of microbial preparations is determined by complex interactions between their biological and technological components. Consequently, both strain selection

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and the physical formulation process are crucial to the development of an effective final product. Various applications of microorganisms have been developed, including microbial seed coating and biostimulants (Bryndina *et al.*, 2019; Muchtar *et al.*, 2014), biopriming and bacterial inoculants (Houida *et al.*, 2022; Roi *et al.*, 2022), and microbial supernatants (Msimbira *et al.*, 2022) to meet different agricultural needs (Park *et al.*, 2018). Spore-forming bacteria, such as those from the *Bacillus* and *Paenibacillus* genera, are particularly beneficial due to their resilience and multifunctional roles in promoting plant growth and protecting crops. Their ability to form spores ensures long-term viability and effectiveness in various agricultural applications, from biofertilizers to biocontrol agents. Spore-forming bacteria are highly resistant to adverse conditions such as heat, drying, and chemicals, which enhances their stability and longevity in agricultural settings (Rowińska *et al.*, 2025a; Thirumurugan and Asha, 2010). Spore formation allows bacteria to survive in dormant states during unfavourable conditions, ensuring their viability when conditions improve. The ability to form spores extends the shelf life of microbial products that contain such type of bacteria, ensuring that they remain effective over extended periods (Bader *et al.*, 2012; Rowińska *et al.*, 2025a; Vishwakarma *et al.*, 2024).

Granulated preparations are of particular interest in the context of large-scale practical applications. Their durability and effectiveness largely depend on the proper selection of a carrier, such as an organic material (e.g. compost or peat), or an inorganic material (e.g. vermiculite, clay, or perlite) which protects the microbial inoculants, enhances their viability, and facilitates their application in the field (Singh and Kumar, 2024). An ideal carrier should meet a range of criteria, including biocompatibility and non-toxicity, provide appropriate moisture conditions, and offer physical protection against environmental factors, while simultaneously enabling the controlled release of microorganisms (Bashan *et al.*, 2014). Despite the growing body of literature on carrier materials for bacterial inoculants, most studies have focused on organic carriers such as peat, talc, or charcoal, or on encapsulation techniques based on alginate and other polysaccharide gels, which protect bacterial cells within a polymeric matrix rather than a granulated mineral structure (Berninger *et al.*, 2018; Vassilev *et al.*, 2020). Comparable long-term storage trials have been conducted on granulated carrier-based bioformulations; for example (Parveen *et al.*, 2023) evaluated microbial viability over 360 days in granules based on talc, charcoal, and gluten mixtures. However, that study focused on organic/mixed carriers and did not address purely mineral clay-type carriers (e.g. bentonite versus chalk) or compare the effect of different moistening and energy-source additives (water, molasses, post-cultivation liquid) on bacterial survival within the granulate. Similarly, review studies on plant growth-promoting bacterial inoculant technology have

repeatedly identified the loss of viability during storage as one of the major unresolved challenges of commercial formulations (Bashan *et al.*, 2014; Kanishka *et al.*, 2025). In the case of spore-forming bacteria, it is crucial to understand the granulation process, the physical properties of the granules, and how these factors impact the stability and germination capacity of the spores. It is also essential to link these data to the physical parameters of the granules, such as compressive strength, solubility, and water absorption, which directly influence the release and colonization success of microorganisms in the soil environment. Without a comprehensive approach, the real application value of granulated biopreparations cannot be fully assessed.

This article aims to comprehensively assess the physical parameters and survivability of biopreparations containing five spore-forming bacteria strains of the genera *Bacillus*, *Paenibacillus*, and *Priestia*, all of which have documented biotechnological potential (Rowińska *et al.*, 2025b).

2. MATERIALS AND METHODS

2.1. Microorganisms

The strains of bacteria used in this study are environmental isolates: *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus velezensis*, *Paenibacillus amylolyticus*, and *Priestia megaterium*. These strains have been identified and their nucleotide sequences are available in GenBank under the genome assembly accession numbers: ASM4051364v1, ASM4051367v1, ASM4051368v1, ASM4051371v1, ASM4051399v1. The characteristics of the strains, such as their enzymatic properties, antagonistic properties, ability to fix atmospheric nitrogen, phosphate solubilization, and cytotoxicity against an insect cell line, were described in our previous work (Rowińska *et al.*, 2025b).

2.2. Granulation

Post-cultivation liquid and spray-dried powder were used for disc granulation. The tested bacterial strains were cultivated in a medium with the following composition: yeast extract (2 g L⁻¹), soy protein concentrate (5 g L⁻¹), calcium chloride dihydrate (0.5 g L⁻¹), glucose (10 g L⁻¹), sodium chloride (0.5 g L⁻¹), magnesium chloride hexahydrate (0.5 g L⁻¹), dipotassium phosphate (1.0 g L⁻¹), iron chloride (0.5 g L⁻¹), manganese sulfate (0.5 g L⁻¹), dissolved in distilled water. The bacteria were cultured for 72 h at 30°C with shaking at 180 rpm (incubator CLW 400 SMART, POL-EKO, Poland, shaker SK-O330-Pro, ChemLand, Poland). Spray-dried powder was obtained for each strain according to the method described in our previous article (Rowińska *et al.*, 2025a) and mixed in equal mass proportions of 1:1:1:1:1. Granules were produced by disc granulation under the following process-apparatus conditions: disc diameter 0.3 m, disc angle 45°, speed 25 rpm. The binding liquid was applied gravitationally *via* pipette. The granules were dried at 60°C for 48 h. Table 1 shows the composition of the granules.

Table 1. Granulate composition

Granulate	Bentonite	Chalk	Bacteria powder	Water	50% molasses	Post-cultivation liquid
	(g)			(ml)		
BBM	191.2	0	8.8	0	75	0
BBW	191.2	0	8.8	100	0	0
CBM	0	239.65	10.35	0	10	0
CBW	0	239.65	10.35	10	0	0
CP	0	239.65	0	0	0	20
BP	191.2	0	0	0	0	100
CW*	0	250	0	10	0	0
CM*	0	250	0	0	10	0
BW*	200	0	0	100	0	0
BM*	200	0	0	0	75	0

*Granulates containing no added microorganisms were used as controls. Abbreviations of granulate names are composed of letters indicating, in order: 1 – the carrier material (B – bentonite, C – chalk); 2 – the microbial inoculum source (where applicable: B – bacteria powder, P – post-cultivation liquid); and 3 – the moistening (M – molasses, W – water).

2.3. Enumeration of microorganisms

The number of vegetative cells was determined by the pour plate method using Tryptic Soy Agar (TSA, Merck-Millipore, Germany). Plates were incubated at 30°C for 24 h (incubator CLW 750 Smart, POL-EKO, Poland). The spore count was determined using the same procedure, with an additional heat-shock treatment before the pour-plate method. Heat shock was performed in a water bath (SUB Aqua Pro, Grant Instruments, England) for 10 min at 80°C, after which the bacteria were cooled in an ice bath. The total number of bacteria was determined by the pour plate method using Tryptic Soy Agar (TSA, Merck-Millipore, Germany) with 0.2% nystatin (Thermo Fisher Scientific, Czech Republic). The plates were incubated at 30°C for 24 h (incubator CLW 750 Smart, POL-EKO, Poland).

2.4. Analysis of the produced granules

2.4.1. Testing of physical parameters

Bulk density and angle of repose were determined. To characterize the shape of the granules, they were analysed according to Zingg's classification. The volumetric proportions of four basic grain shapes-disc-shaped, spherical, bladed and rod-like-were determined using a Kamika analyser AWK 3D (Poland). The mechanical strength of the obtained granules after drying was also evaluated. Their compressive strength was determined using an INSTRON testing machine (USA) by measuring the mean force required to crush a single granule (N). Ten granules of the most abundant size and shape were tested.

2.4.2. Survivability of microorganisms during storage

Granules were stored in a plastic container (1.2 L volume) at room temperature (21±2°C). Vegetative cells and spores were enumerated over a 12-month storage period (enumeration at months 0, 2, 4, 6, and 12).

2.4.3. Disintegration of granules and release of microorganisms

2.4.3.1. Model conditions

Based on physical parameters and survivability, 4 granulates (2 with bentonite and 2 with chalk) were chosen for further studies. Disintegration of granules was assessed in distilled water and during a field trial. Six independent replicate samples were prepared, each containing 1 granule from the most abundant shape and size fraction, based on previous physical analyses. The granules were placed in plastic containers (100 mL capacity) containing 50 mL of distilled water. Visual observations were conducted at pre-determined time intervals: 1, 15, 30, 60 min, 2, 3, 4, 8, 20, 28, 32 h. All trials were conducted without stirring. The following parameters were systematically documented: the granule disintegration rate, based on the temporal pattern of visible granule loss (complete, partial, or absent); changes in granule morphology, including softening, cracking, and fragmentation; medium coloration; medium turbidity; and sediment formation.

2.4.3.2. Field conditions

A field trial was conducted to assess the disintegration of granules in soil under environmental conditions. In Poland, spring rye is typically sown in early spring, around March to April (Kadłubiec and Bojarczuk, 2003), while maize is

usually sown between April and May (Gąsiorowska *et al.*, 2009; Rosa, 2013). Therefore, based on sowing practices, weather forecasts, and the assumption that granular fertilizer would need time to dissolve in the soil, we decided to conduct a field trial starting March 11th 2025. The field trial was conducted in Zduńska Wola (Łódź voivodship, Poland). Temperature during field trial ranged from -1.9 to 17.5°C and relative humidity from 33 to 85% (Fig. S1) representing varying conditions in March. The granules were incorporated into the soil at an application rate of 300 kg ha^{-1} within a $50 \times 50\text{ cm}$ garden plot located in Zduńska Wola, Łódź voivodship. The temperature was measured at soil level in the wooden box using a ThermoPro TP357 (China). Granules were observed visually and soil samples were taken for microbial enumeration. Samples were taken after rain and once a week if there was no rain. Samples were taken from 3 different places in the soil, avoiding the visually seen granules. Total bacterial counts and spore-forming bacterial counts were determined.

After four weeks, the experiment was terminated because no granules remained visible. The soil was raked, and samples were collected for the final enumeration.

2.5. Statistical analyses

Statistically significant differences were determined using ANOVA and a post-hoc Tukey's test ($p < 0.05$). Grubbs' test was applied to remove outliers from the mechanical-strength data, resulting in a cleaned dataset of 5 values. Statistical analyses were performed using RStudio, v. 2025.05.1+513 "Mariposa Orchid" Release (ab7c1bc-795c7dceff8f26215b832a3649a19fc16c, 2025-06-01).

3. RESULTS AND DISCUSSION

3.1. Physical parameters of granules

The volumetric analysis revealed differences in granule size depending on their composition. The bentonite-based formulations BBM (Fig. S2a) and BBW (Fig. S2b) exhibited similar, broad, distributions with maxima in the $4\,500\text{--}6\,000\text{ }\mu\text{m}$ range (5.0 and 4.0% volume, respectively), indicating that molasses had little influence on granule size when bacteria were present. A comparable similarity was observed between the chalk-based formulations CBM (Fig. S2c) and CBW (Fig. S2d), both showing narrower distributions strongly shifted toward smaller diameters, with maxima around $1\,000\text{--}1\,500\text{ }\mu\text{m}$ (8.1 and 7.0% volume, respectively) and a sharp decline above ca. $3\,000\text{ }\mu\text{m}$. When post-cultivation liquid was used instead, the differences between carriers became less pronounced: CP (Fig. S1e) and BP (Fig. S1f) both displayed broad, multimodal distributions with local maxima between $5\,500\text{--}6\,000\text{ }\mu\text{m}$ and $7\,000\text{--}9\,000\text{ }\mu\text{m}$, and similar peak volumes (4.3 and 3.9%, respectively), suggesting that the composition of the post-cultivation liquid had a stronger effect on granule structure than the carrier itself. In granules without bac-

teria, the differences between formulations prepared with water and those prepared with 50% molasses were more marked. For the chalk carrier, CW (Fig. S2g) showed a broader, less regular distribution, whereas CM (Fig. S2h) exhibited a narrower and more homogeneous distribution. A similar pattern was found for the bentonite carrier, where BW (Fig. S2i) noted wider spread of larger particles above $10\,000\text{ }\mu\text{m}$, while BM (Fig. S2j) showed a more concentrated distribution. These results indicate that in the absence of bacteria, the use of molasses rather than water led to more uniform granule populations for both carriers. The target granule size was up to 10 mm in diameter, as granules exceeding 10 mm are often considered oversized for fertilizer applications (Siuda *et al.*, 2018). Most formulations fell within this range. Granules measuring around $2.0\text{--}5.5\text{ mm}$ have been found to have high static strength and are suitable for continuous application in agriculture (Skovorodnikov and Cherepanova, 2019). Similarly, fertilizer particles with average diameters of around $3.68\text{--}4.29\text{ mm}$ enable precise dosing and effective nutrient delivery to plants (Lillerand *et al.*, 2021). The CBM and CBW formulations produced smaller granules, predominantly up to 5 mm in diameter, than the bentonite-based formulations.

The shapes of the granules were analysed according to Zingg's classification. Spherical and rod-like shapes were the most abundant. For the bentonite-based formulations, spherical granules accounted for over half of the particle volume in BBM (63.1%, Fig. S3a) and almost half in BBW (47.6%, Fig. S3b), with the highest volume fractions concentrated in the upper-right region of the plot (ratio of minimum to intermediate diameter and intermediate to maximum diameter). A similar predominance of spherical shapes was observed for BW (54.5%, Fig. S3i) and BM (50.0%, Fig. S3j), indicating that bentonite-based granules, irrespective of the liquid used, tended to develop more regular morphologies. Among the chalk-based formulations, CBW (61.3%, Fig. S3d) likewise showed spherical granules constituting more than half of the particle volume, whereas CBM (spherical: 47.6%; rod-like: 42.0%, Fig. S3c) exhibited a more even distribution between the spherical and rod-like regions, without a single morphology clearly exceeding half of the total volume. The chalk formulation prepared with water, CW (59.3%, Fig. S3g), was distinct in that rod-like granules constituted more than half of the particle volume, with the highest-intensity intervals located in the lower-right part of the plot (low intermediate-to-maximum diameter ratio, high minimum-to-intermediate diameter ratio). In contrast, CM (spherical: 35.9%; rod-like: 47.5%, Fig. S3h) displayed a more balanced contribution of spherical and rod-like fractions. For the granulates prepared with post-cultivation liquid, BP (66.0%, Fig. S3f) showed a clear predominance of spherical granules, whereas CP (spherical: 47.8%; rod-like: 43.0%, Fig. S3e) exhibited a more mixed distribution, with substantial contributions from both the spherical and

rod-like regions and no single morphology reaching a majority share. Taken together, these results suggest that carrier type had a stronger and more consistent influence on granule shape than the type of granulation liquid or the presence of bacteria powder. The shape of fertilizer granules significantly influences their physical and mechanical properties, affecting how they are spread and their interaction with the environment. Spherical or near-spherical shapes are often preferred for their uniformity and ease of handling, which can lead to more consistent application rates (Jotautiene *et al.*, 2025; Zhang *et al.*, 2021). These shapes also enhance flowability and reduce the likelihood of clogging in spreading equipment (Maroof *et al.*, 2020).

The humidity, bulk density, angle of repose, and compressive strength of the granules were assessed. Humidity was predominantly influenced by the carrier material, with bentonite-based granules exhibiting significantly higher values than their chalk-based counterparts. Within granules formulated with identical carriers, notable variations in humidity emerged depending on the binding agent employed, such as water, molasses, or post-cultivation liquid solutions. The highest humidity was recorded for granulates BBW (65.37) and BW (62.42), which did not differ significantly from one another (Fig. 1a). Similarly, no statistically significant difference was detected between granulates BBM and BM (Fig. 1a), indicating that addition of bacterial powder did not substantially affect humidity in bentonite-based formulations. However, the selection of

binding agent significantly influenced this parameter. In contrast, granulates CBM, CBW, CW, and CM exhibited the lowest moisture content, ranging from 3.77 to 4.57% without statistically significant differences. Granulate CP demonstrated comparatively elevated humidity at 8.56% (Fig. 1a). Bentonite is recognized for its excellent water adsorption properties, which are primarily due to its high montmorillonite content (Castrillo *et al.*, 2018). The addition of bentonite to granulates can significantly enhance their moisture retention capabilities. This is particularly beneficial in processes like pelletizing, where maintaining optimal moisture levels is crucial for achieving the desired mechanical properties (Obraniak *et al.*, 2018; Zhuravlev *et al.*, 2020). Generally, a lower humidity percentage is associated with better strength and stability, although specific target values differ across applications (Siuda *et al.*, 2021). For microbial fertilizers, maintaining a balance between moisture content and microbial viability is essential. High temperatures and low humidity during drying can reduce the microbial population in organic fertilizers, affecting their functional efficacy (Sahwan *et al.*, 2016). For example, a study of *Metarhizium humeri microsclerotia* found that a relative humidity of around 91% was beneficial for conidial production when glycerine was used as a humectant, indicating that higher humidity levels can support

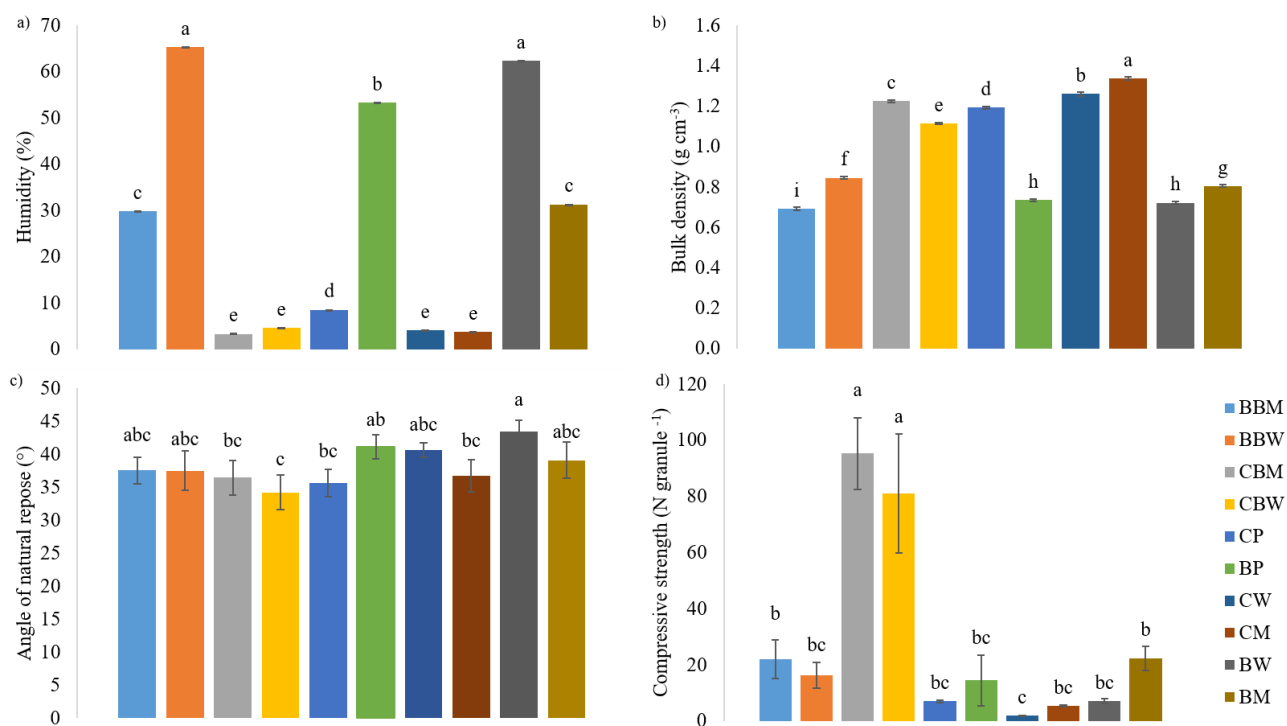


Fig. 1. Physical parameters of granulates: a) humidity, b) bulk density, c) angle of repose, d) compressive strength. Different letters indicate statistically different groups (Tukey's test $p < 0.05$).

microbial activity and sporulation. However, excessive humidity can lead to agglomeration and reduced efficacy (Catão *et al.*, 2021).

Bulk density also depended significantly on the carrier, with granulates based on chalk having the highest bulk density, ranging from 1.34 g cm⁻³ (granulate CM) to 1.12 (granulate CBW). Granulates based on bentonite showed lower bulk density, ranging from 0.85 (granulate BBW) to 0.69 (granulate BBM) (Fig. 1b). The bulk density of microbial preparations can be affected by particle size distribution, moisture content, and the presence of voids or air pockets within the material (Gužela *et al.*, 2024). The bulk density of granular fertilizers often ranges from 0.72 g cm⁻³ (for prilled urea) to 1.2 g cm⁻³ (for muriate of potash (KCl)) (Fulton, 2025), which are a similar values to those obtained for the granules in our study. While granules with higher bulk density offer logistical advantages such as better space utilization and reduced packaging costs (Braga *et al.*, 2018), excessively high bulk density may compromise flowability and increase caking tendency, requiring optimization to balance economic benefits with handling properties (Vinayak *et al.*, 2024).

The angle of repose was similar for all the tested granulates, with few statistically significant differences. Values ranged from 43.44° for BW to 34.22° for CBW (Fig. 1c). A low angle of repose is beneficial for storage and handling (Rostocki *et al.*, 2024). According to Carr's classification of powder flowability based on the angle of repose, the granulates can be classified as either free-flowing or as having fair-to-passable flowability, indicating their suitability for gravity-driven transport (Beakawi Al-Hashemi and Baghabra Al-Amoudi, 2018).

Compressive strength was assessed using granules selected from the most abundant fraction based on the previously determined shape and size distributions. Granulates CBM and CBW, based on chalk and bacteria powder, showed the highest compressive strength (95.30 and 81.00 N). Granules with the addition of 50% molasses exhibited higher compressive strength. Similarly, granules based on bentonite showed higher strength with the addition of

bacteria powder and 50% molasses than with water or post-cultivation liquid (granulate BBM – 22.00 N) (Fig. 1d). The control granulate without bacteria powder exhibited significantly lower compressive strength than the granules with bacteria powder, which may be attributed to the physical properties of the bacteria powder and post-cultivation liquid. The addition of molasses increased the compressive strength of the control granules by 3.38 N for chalk-based granules and by 15.20 N for bentonite-based granules compared with the corresponding granules prepared using water. The force that fertilizer granules can withstand without structural damage should be at least 10 N (Ławińska *et al.*, 2020). The tested granules containing bacteria passed this requirement. The choice of binder affects the compressive strength of the final granules. Siuda *et al.* (2021) showed that molasses can be used as a binder to obtain granules with satisfactory quality parameters (Siuda *et al.*, 2021). Moreover, the concentration of molasses can influence compressive strength. Ławińska *et al.* (2020) showed that granules wetted with a 33% aqueous solution of molasses had a compressive strength below 10 N, while using a 66% aqueous solution of molasses produced granules with a compressive strength above 10 N (Ławińska *et al.*, 2020). In our study, the 50% molasses solution that was used also proved effective in combination with bacteria powder.

3.2. Survivability of microorganisms during storage

The survivability of bacteria was assessed over a 12 month period. The number of bacteria fluctuated, which may be due to uneven distribution in the granules (Fig. 2, Table S1). Granulates based on bacteria powder showed a statistically significant higher number of bacteria than granulates based on post-cultivation liquid. However, all the granulates exhibited high survivability over time. At month 12 of storage, the lowest decrease in the number of bacteria was noted for granulate CBW (0.04 log CFU mL g⁻¹ for vegetative cells and 0.03 log CFU mL g⁻¹ for spores).

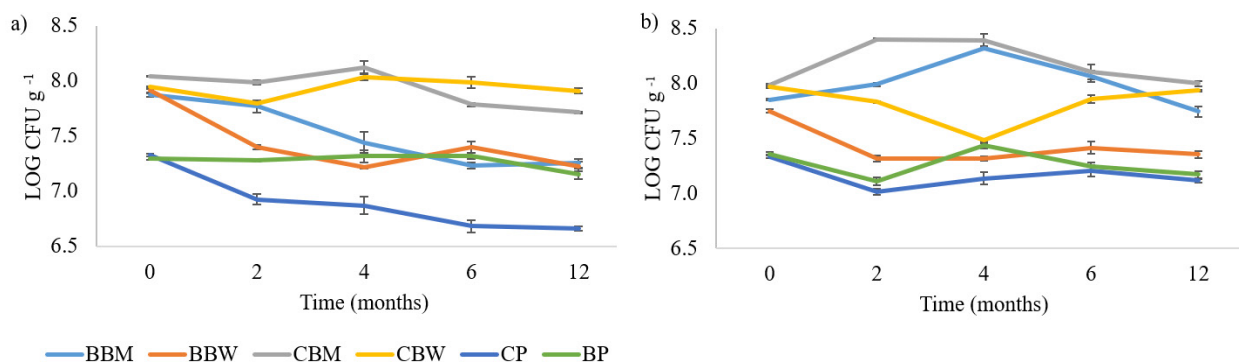


Fig. 2. Survivability of microorganisms during storage: a) vegetative cells, b) spores.

The largest decrease was observed for granulate CP ($0.67 \log \text{CFU mL}^{-1}$ for vegetative cells and $0.22 \log \text{CFU mL}^{-1}$ for spores) (Fig. 2, Table S1).

The survivability of the microorganisms in granulates based on bacteria powder was similar to the previously tested survivability of microorganisms after spray-drying (Rowińska *et al.*, 2025a). In that study, the greatest decrease in vegetative cell count was noted for *P. amylolyticus* after two months of storage ($0.5 \text{ LOG CFU g}^{-1}$). Spore numbers were more stable, as only *B. velezensis* showed a decrease during storage over the 12 months period. The greater decreases in bacteria counts observed in the current study may be a result of additional heat usage during the granule-preparation process. Differences in microbial survival between granulates prepared with bacterial powder and those prepared with post-cultivation liquid may be attributed to the protectants used during spray drying (Rowińska *et al.*, 2025a), as no protectants were added during granulation. Protective substances, such as skimmed milk or sugars, may be added during spray drying to increase cell viability (Kakuda *et al.*, 2023).

Microbial survival in granulated formulations has not been extensively studied, although previous research has highlighted the potential advantages of this formulation type. For example, a granular formulation of *Serratia entomophila* has been developed to improve the shelf-life and storage characteristics of this bacterium, which is used as a microbial control agent against the New Zealand grass grub. A comparison of the granular and liquid formulations showed that high bacterial counts were maintained in the granules throughout the experiment. Moreover, *S. ento-*

mophila populations remained stable for up to five months in soil inoculated with the granules across a range of soil moisture levels (O'Callaghan and Gerard, 2005). The use of thermal processing can further help reduce pathogen loads in organic fertilizers, improving their safety and efficacy. Granular fertilizers are dried at increased temperatures and can include organic substances as binding agents (Chen and Jiang, 2014; Sarbani and Yahaya, 2022).

3.3. Disintegration of granules and release of microorganisms

Based on their physical properties and bacterial survival, two bentonite-based granulates (BBM and BP) and two chalk-based granulates (CBM and CBW) were selected for the disintegration assessment. Table S2 presents the visual changes observed during their disintegration in distilled water. Marked differences were observed in the disintegration of chalk-based and bentonite-based granules. Granules CBM and CBW cracked and dissolved much more rapidly, dissolving completely within approximately 2 h of immersion in distilled water, whereas BBM and BP granules absorbed water and dissolved significantly more slowly. Granules BBM dissolved after approx. 28 h and granules BP dissolved after 20 h (Table S2). Similarly to their dissolution in distilled water, the BBM and BP granules differed from the CBM and CBW granules in their response to rainfall. After the first rainfall event on day 2, visible changes were observed in the granules. The bentonite-based granules increased in volume, whereas the chalk-based granules partially dissolved (Fig. 3). Granule dissolution increased following each rainfall event. Samples were collected and



Fig. 3. Visual comparison of granules: a) before and b) after rain during field trial.

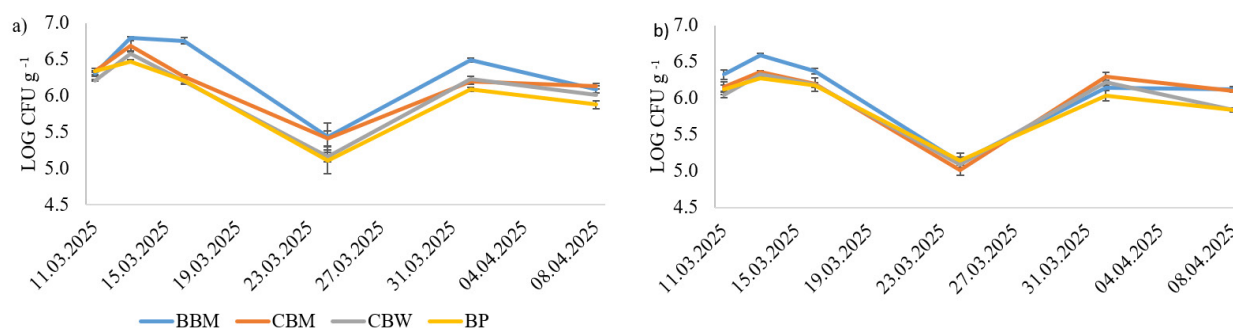


Fig. 4. Number of bacteria in the soil: a) vegetative cells, b) spores.

the granules were examined on the day after each rainfall event. After 28 days, no visible granule residues remained, and the field trial was therefore terminated.

The observed differences in the disintegration of granular biopreparations depending on the carrier used – bentonite or chalk – are consistent with previous reports. Other studies have shown that bentonite can be an effective carrier for slow-release fertilizers, improving nutrient retention (Rasse *et al.*, 2022). Several studies have demonstrated that bentonite-based slow-release fertilizers improve nutrient use efficiency, reduce leaching losses, and enhance crop growth, supporting environmental sustainability and agricultural productivity (Hussain *et al.*, 2022; Mustaffa *et al.*, 2024; Rashid *et al.*, 2023). Chalk- and limestone-based carriers are effective sources of calcium but are less versatile for controlling the release of other nutrients; therefore, particle-size optimization is often required to regulate their dissolution (Farahat *et al.*, 2016; Loide *et al.*, 2018).

The bacterial count in the soil changed over the course of the trial (Fig. 4). Although the addition of granules increased the total number of bacteria in the soil, the BBM granulate produced a more prolonged effect, which may be attributed to its lower solubility. The highest numbers of vegetative cells and spores in the soil were recorded on March 13th and 16th. At the end of the experiment, there were no statistically significant differences between granulates BBM, CBM, and CBW. Soil treated with granulate BP showed the lowest number of bacteria (Fig. 4, Table S3). Moreover, bacterial counts were strongly influenced by temperature conditions. In all trials, the number of bacteria significantly fell between March 16th and March 24th after the temperature lowered to -0.7°C on March 16th and -1.9°C at March 18th (Fig. S1, Fig. 4, Table S3).

The number of bacteria in soil depends on atmospheric conditions. Bacterial growth tends to decrease at freezing temperatures, with a shift in the microbial community favouring fungi over bacteria. This results in an increased

fungal-to-bacterial growth ratio under freezing conditions (Haei *et al.*, 2011). Freeze-thaw cycles can lead to shifts in bacterial community structure, with some species being more affected by these cycles than others. However, the overall impact on bacterial functional capabilities may be minor (Juan *et al.*, 2018).

4. CONCLUSIONS

The survival of microorganisms in microbiological biopreparations during storage is crucial for their effective use in agriculture. This study has demonstrated that granulated biopreparations containing spore-forming bacterial strains *Bacillus subtilis*, *Bacillus velezensis*, *Bacillus licheniformis*, *Priestia megaterium*, and *Paenibacillus amylolyticus* can be stored without significant loss of viable bacteria for 12 months. The highest survival rate was observed for granulates containing spray-dried bacteria. The use of protective agents during granulation should be investigated further to enable the direct use of post-culture liquid without additional processing steps, such as spray drying, while maintaining the desired level of microbial survival.

We found that the physical properties of the granulated biopreparation depend primarily on the choice of carrier and binder. The granules containing molasses generally exhibited higher compressive strength. The presented results show that bentonite- and chalk-based granules differ primarily in disintegration time, as bentonite absorbs water excessively, which can lead to a slower release of microorganisms. In the context of microbial biopreparations, this may represent an advantage under changing environmental conditions; however, further research is needed to establish appropriate guidelines for their use by farmers.

This study provides important data necessary to optimize production, ensure quality, and predict the effectiveness of biopreparations in model and field conditions.

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Figure 3 was created in <https://BioRender.com>

Conflict of interest: The authors declare that they have no conflict of interest.

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