International Journal of Biological Macromolecules 320 (2025) 146082

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Microencapsulation of *Bacillus subtilis* by spray-drying using starch hydrolysates with different dextrose equivalent values

Marina Momesso Lopes [a,b,c], Cristiane Sanchez Farinas [b,c,*], Manuel Martínez Bueno [d,e], Pedro J. García-Moreno [a,**], Emilia M. Guadix [a,e]

[a] Department of Chemical Engineering, University of Granada, Granada, Spain
[b] Nanotechnology National Laboratory for Agriculture, Embrapa Instrumentation, São Carlos, SP, Brazil
[c] Graduate Program of Biotechnology, Federal University of Sao Carlos, São Carlos, SP, Brazil
[d] Department of Microbiology, University of Granada, Granada, Spain
[e] Institute of Biotechnology, University of Granada, Granada, Spain

ARTICLE INFO

Keywords:
Bacillus
Encapsulation
Spray-drying
Starch hydrolysates
Carbohydrates

ABSTRACT

Sustainable agricultural practices require innovative solutions to enhance productivity while reducing environmental impact. The use of plant growth-promoting microorganisms, such as *Bacillus subtilis*, as biofertilizers is a promising strategy. However, ensuring cell viability during storage and under field conditions remains a challenge. This study investigates the encapsulation of *B. subtilis* via spray-drying using starch hydrolysates with different dextrose equivalent (DE) values (DE-8, DE-18, and DE-38) as wall materials. Encapsulation efficiency was approximately 80 % for all formulations. The DE values influenced microcapsule morphology and cell release profiles, with higher DE materials producing smoother, smaller, and more homogeneous particles. The microcapsules effectively protected cells against high salinity and acidic pH stresses. Thermal stability was significantly improved with DE-18 and DE-38, maintaining over 95 % viability after 72 h at 50 °C. Under UV exposure, DE-18 demonstrated superior protection. Storage stability tests confirmed enhanced longevity for encapsulated cells compared to free bacteria, with higher DE microcapsules demonstrating better resilience to elevated temperatures. These findings highlight the potential of starch-based microencapsulation to improve biofertilizer performance, ensuring microbial survival and efficacy in diverse environmental conditions.

1. Introduction

The challenge of enhancing agricultural productivity to meet the growing global food demand is increasingly urgent. Factors such as climate change, declining soil fertility, and limited arable land often lead to the excessive use of agrochemicals to achieve higher crop yields. However, the intensive use of these chemicals poses significant risks to both human health and the environment [1,2]. Biofertilizers, particularly those based on beneficial microorganisms such as *Bacillus* species, offer a promising solution for improving crop yields in an environmentally sustainable manner [3,4].

Bacillus strains are well known for their ability to enhance plant growth through multiple mechanisms, including nutrient solubilization, phytohormone production, and biocontrol against phytopathogens. A key feature of many *Bacillus* strains is their ability to solubilize phosphorus, an essential nutrient for plants, positioning them as strong candidates for use as biofertilizers [5–7]. Despite their potential, the preservation of *Bacillus* cell viability during storage and under challenging field conditions remains a barrier to their commercialization [2,8]. Moreover, the development of biofertilizers with controlled release mechanisms is highly desirable, as such formulations could promote sustained plant growth without the need for repeated applications [2,9,10].

Encapsulation by spray-drying emerges as a promising technique to protect and maintain the viability of *Bacillus* cells under adverse conditions during production, transportation, storage and application, as well as enabling controlled release profiles [9,11]. Spray-drying is widely used in various industries due to its efficiency, cost-effectiveness, rapid processing times, and scalability [12–15]. Studies focusing on encapsulation of *Bacillus* strains using spray-drying for agricultural

* Corresponding author.
** Correspondence to: P.J. García-Moreno, Department of Chemical Engineering, University of Granada, Granada, Spain.
E-mail addresses: cristiane.farinas@embrapa.br (C.S. Farinas), pjgarcia@ugr.es (P.J. García-Moreno).

https://doi.org/10.1016/j.ijbiomac.2025.146082
Received 19 March 2025; Received in revised form 14 June 2025; Accepted 14 July 2025
Available online 15 July 2025

applications have demonstrated promising results in terms of maintaining cell viability during storage and in field conditions [16,17].

The success of spray-drying encapsulation depends on the choice of encapsulating agents, which must meet specific criteria, including appropriate glass transition temperatures, favorable drying characteristics, and the ability to form solutions with low viscosity at high solid concentrations. These properties facilitate efficient atomization and reduce drying energy costs [9,18]. For agricultural applications, the encapsulating material should be low-cost, readily available, non-toxic, biodegradable, and capable of effectively protecting the encapsulated cells [9,19,20].

Starch hydrolysates, which are digestion products of starch, are promising candidates as encapsulating materials due to their ability to meet these criteria. Additionally, starch hydrolysates enhance cell adhesion, act as carbon source and thus have been widely used as wall materials for encapsulation of microorganisms by spray-drying. The dextrose equivalent (DE) value of starch hydrolysates influences the properties of the resulting microcapsules, including their morphology and release characteristics [18,21]. However, the impact of different DE values on the properties of encapsulated *Bacillus* strains has not been explored yet in the context of biofertilizer applications.

The aim of this study was to investigate the use of starch hydrolysates with different DE values (DE-8, DE-18, and DE-38) as wall materials for encapsulating *Bacillus subtilis* via spray-drying. Specifically, this study aimed to: (1) evaluate the morphological properties of the microcapsules, (2) assess the viability of the encapsulated cells after the spray-drying process and investigate their growth and release behaviors, (3) examine the maintenance of cell viability under stress conditions, and (4) assess cell viability during storage using the Accelerated Shelf-Life Test (ASLT) method. This work provides new insights into the influence of starch hydrolysate DE values on microbial encapsulation by spray-drying, with the potential to enhance the development of effective biofertilizers for agricultural applications.

2. Methodology

2.1. Materials

Starch hydrolysates of different DEs were used as wall material for the spray-drying encapsulation processes: DE-8 (C*Dry™ MD 01955) and DE-18 (C*Dry™ MD 01915), both classified as maltodextrins (DE values <20), and DE-38 (C*Dry™ GL 01934) classified as glucose syrup (DE value >20). These materials were kindly donated by Cargill (Barcelona, Spain). All other reagents were of analytical grade.

2.2. Microbial cultivation

Bacillus subtilis ATCC 6051a was encapsulated by spray-drying for the development of the biofertilizer. The bacterial strain was first reactivated in Tryptic Soy Broth (TSB) medium in 500 mL Erlenmeyer flasks and incubated at 30 °C, 250 rpm for 48 h. Following incubation, the culture was centrifuged at 8,000 rpm, 25 °C in Falcon tubes until visible cell precipitation was achieved. The supernatant was then discarded, and the resulting cell pellet was resuspended and concentrated in a 0.85 % saline solution. To determine bacterial viability, an aliquot of the suspension was plated, and the number of viable cells (log CFU mL^{-1}) was quantified using the plate count method. Based on these results, the required volume of cell suspension for each encapsulation formulation was calculated. For the spray-drying process, the inoculum volume was adjusted to achieve a final bacterial concentration of 9 log CFU g^{-1} dry matter in the microcapsules. Previously, the proportion of vegetative cells and spores in the culture was determined by comparing CFU/mL counts before and after shock treatment (30 min, 62 °C). The results indicated that nearly the entire culture used for encapsulation consisted of spores.

2.3. Production of microcapsules by spray-drying

For the encapsulation process, solutions were prepared by dissolving starch hydrolysates (DE-8, DE-18, or DE-38) at a concentration of 40 % (*w/v*) in distilled water under continuous stirring at 540 rpm overnight at room temperature. After complete dissolution, the calculated volume of bacterial suspension was added, stirred for 10 min, and immediately subjected to the spray-drying process. Spray-drying was performed using a laboratory-scale Büchi B-190 spray dryer (Büchi Labortechnik, Flawil, Switzerland) with an inlet temperature of 140 °C and an outlet temperature of 80 °C. The drying air flow rate was maintained at 25 $Nm^3\ h^{-1}$. The resulting dried microcapsules were designated as MC-8, MC-18, and MC-38, corresponding to formulations prepared with starch hydrolysates of DE-8, DE-18, and DE-38, respectively. To determine the spray drying process yield, which is related to powder recovery, the collected microcapsules were weighed, and the yield percentage was calculated based on the initial solid content in the feed solution and the final mass of the dried microcapsules.

2.4. Morphological characterization of microcapsules

The morphology of the microcapsules was investigated via scanning electron microscopy (SEM) using an FESEM microscope (LEO 1500 GEMINI, Zeiss, Germany). A thin layer of microcapsules was placed on the carbon tape and carbon-coated using an EMITECH K975X Turbo-Pumped Thermal Evaporator (Quorum Technologies, UK). The micrographs were taken at different magnifications (500, 2000 and 5000×). The images were then analyzed using the ImageJ software (National Institute of Health) and > 200 randomly selected microcapsules were measured to determine the particles size distribution.

2.5. Encapsulation efficiency

To assess encapsulation efficiency, 0.05 g of microcapsules was dispersed in 5 mL of 0.85 % saline solution using vortex agitation to release the encapsulated cells. An aliquot of the resulting suspension was plated, and viable cell concentration (log CFU g^{-1}) was determined using the plate count method. Encapsulation efficiency was expressed as a percentage, where 100 % corresponds to the initial bacterial concentration (9 log CFU g^{-1}) added at the beginning of the process, as described in Section 2.2, and compared to the viable cell concentration obtained after the assay described in this section. All assays were performed in triplicate to ensure reproducibility.

2.6. Cell release and growth assays

2.6.1. Cell release and growth on solid medium

To evaluate cell release behavior and growth of free and encapsulated *Bacillus subtilis*, TSA plates were prepared with different treatments. The treatments included: (i) a cell suspension corresponding to 5 log CFU (treatment B); (ii) the same volume of cell suspension combined with 0.01 g of starch hydrolysates used in the encapsulation process (B + DE-8, B + DE-18, and B + DE-38); and (iii) 0.01 g of microcapsules (MC-8, MC-18, and MC-38), also corresponding to approximately 5 log CFU. Plates were incubated, and images were captured at 6, 24, and 48 h. The halo size (mm) formed around each treatment was measured using ImageJ software (National Institutes of Health, USA) to assess bacterial growth and release patterns. The data were then used to construct graphical representations of the results.

2.6.2. Cell release and growth in wet environment

To assess cell release behavior and growth in a wet environment over time, 0.85 g of microcapsules was suspended in 8.5 mL of 0.85 % saline solution and stirred at 250 rpm, 30 °C. Aliquots were collected at 10 min, 30 min, 1 h, 3 h, 6 h, 24 h, 48 h, and 72 h, followed by plating to determine viable cell concentration (log CFU g^{-1}) using the plate count

method. All experiments were conducted in triplicate to ensure reproducibility.

2.7. Maintenance of cell viability under stress conditions

The experiments aimed to evaluate cell viability maintenance of free and encapsulated *Bacillus subtilis* under stress conditions of high salinity and acidic pH. For salinity stress, 450 μL of free bacteria and 0.45 g of microcapsules were suspended in 4.5 mL of 50 % saline solution and stirred at 250 rpm, 30 °C. For pH stress, the same amounts were suspended in 0.85 % saline solution, adjusted to pH 2 using 1 M HCl, and subjected to the same stirring conditions. Aliquots were collected at 30 min, 6 h, and 24 h, followed by plating to determine viable cell concentration (log CFU) using the plate count method. Viability results were expressed as a percentage, where 100 % corresponds to the viability of free bacteria and the post-encapsulation viability of each microcapsule formulation. All experiments were conducted in triplicate to ensure reproducibility.

To evaluate the effect of heat and UV exposure on cell viability, free and encapsulated *Bacillus subtilis* were subjected to thermal and ultraviolet stress conditions. For heat exposure, samples were incubated at 50 °C (relative humidity of 20 %) for 24 h and 72 h. For UV exposure, free and encapsulated cells were exposed to UV radiation (Esco Technology, 15 A, model U319) (at 25 °C and 45 % of relative humidity) for 5 min and 20 min. After treatment, free bacteria were directly plated, while microcapsules were solubilized in 0.85 % saline solution using a vortex before plating. Viability results were expressed as a percentage, where 100 % corresponds to the viability of free bacteria and the post-encapsulation viability of each microcapsule formulation. All experiments were conducted in triplicate to ensure reproducibility. Furthermore, for the sake of comparison, the assays evaluating free and encapsulated cells were conducted at the same time.

2.8. Cell viability at storage

An Accelerated Shelf-Life Test (ASLT) was performed to estimate the cell survival of the free bacteria and encapsulated cells during storage, as described in Lopes et al. [22]. The products were stored at temperature values of 4, 30, and 50 °C with relative humidity of 60, 40 and 20 %, respectively. At each time point of 0, 7, 14, 21 and 28 days, 100 μL of the free bacteria and 0.015 g of the microcapsules were collected. Free bacteria were plated, while the microcapsules were dispersed in 150 μL of 0.85 % saline solution using a vortex before plating. All assays were performed in triplicate. Furthermore, for the sake of comparison, the assays evaluating free and encapsulated cells were conducted at the same time. The spreadsheets and calculations of this assay are detailed in the Supplementary Material 1.

After obtaining the CFU data, the estimated time from the ASLT was evaluated by a first-order degradation kinetics (Eq. 1):

$$lnN_t - lnN_0 = -kt \tag{1}$$

Where N_0 and N_t are the initial and final cell concentrations (CFU) respectively, and k is the degradation rate constant (day^{-1}).

However, most ASLT studies depicted that the Arrhenius Equation is the most common way to describe the deterioration rate as a function of the temperature (Eq. 2):

$$lnk = lnk_0 - \frac{E_a}{RT} \tag{2}$$

where k is the reaction rate constant, k_0 is the Arrhenius constant, E_a is the activation energy ($J\,mol^{-1}$), R is the universal gas constant ($8.314\,J\,K^{-1}\,mol^{-1}$) and T is the reaction temperature (K). After determining the necessary parameters, the shelf-life time (t) of the materials can be determined by the Eq. 3, where k_T is the calculated value of k for each temperature, and A_0 and A_t are the initial and final cell concentrations (CFU), respectively.

$$t = \frac{ln_{Ao} - ln_{At}}{k_T} \tag{3}$$

2.9. Statistical analysis

Bacterial concentrations (CFU) were log-transformed (log CFU) to improve homogeneity of variances. Error bars of the figures were represented by the mean standard deviation (±SD) of the replications. Data of cell viabilities and size of halos were subjected to analysis of variance (ANOVA) and Tukey's test (95 % of confidence level) performed using Origin v. 9.8 software.

3. Results and discussion

3.1. Characterization of the microcapsules

The relationship between DE value and feed solution characteristics is illustrated in Fig. 1, which depicts key structural and physicochemical differences among the starch hydrolysates used to produce the microcapsules by spray-drying. For instance, the processing yield of the spray-drying encapsulation process showed a clear dependence on the dextrose equivalent (DE) value of the starch hydrolysates used as encapsulating agents. The highest yields were obtained for MC-18 (21.1 %) and MC-38 (20.4 %), whereas MC-8 exhibited a yield of 11.7 %. These differences can be attributed to the viscosity of the feed solutions, which is directly influenced by the molecular structure of the hydrolysates (Fig. 1). High viscous solutions tend to form irregular atomized droplets, which reduce drying efficiency and result in lower process recovery. Therefore, the higher viscosity of MC-8 likely explains its significantly lower yield. The observed trend is consistent with previous studies that report a direct relationship between solution viscosity and spray-drying yield [23,24].

The SEM images presented in Fig. 2 reveal distinct morphological differences among the microcapsules produced with starch hydrolysates of varying dextrose equivalent (DE) values. The MC-38 microcapsules exhibited a smooth, spherical, and uniform surface with minimal structural defects. This is attributed to the higher proportion of low-molecular-weight sugars present in DE-38, which can act as plasticizers, enhancing matrix flexibility and preventing surface shrinkage during drying [25,26]. In contrast, the MC-18 microcapsules displayed intermediate surface characteristics, with moderately smooth surfaces interspersed with minor wrinkles. The MC-8 microcapsules, however, showed rough, irregular surfaces with noticeable cracks and shrinkage. The lower DE value corresponds to a higher presence of long-chain saccharides, which reduce chain mobility and elasticity, leading to uneven drying and structural collapse.

The particle size distribution (Fig. 2D) further highlights the impact of DE values on microcapsule formation. The MC-38 microcapsules had the smallest and most uniform particle sizes, primarily ranging from 5 to 20 μm. This uniformity is attributed to the lower viscosity and enhanced solubility of DE-38, which facilitates the formation of smaller, evenly distributed droplets during atomization. Such smaller particles can be beneficial for agricultural applications, ensuring better dispersion of the product [27].

The MC-18 microcapsules exhibited intermediate size distribution, showing that moderate DE value allows for a balance between viscosity and atomization efficiency. In contrast, the MC-8 microcapsules had the largest and most heterogeneous size distribution. The higher viscosity of the DE-8 solution likely hindered effective atomization, producing larger droplets that dried into larger, less uniform microcapsules [13,18].

As observed in the SEM images, the surface of the microcapsules is

Fig. 1. Important information on the characteristics of the different DE starch hydrolysates, which influence the properties of the microcapsules produced by spray-drying.

clean of free bacteria, indicating they were microencapsulated or eliminated by thermal degradation during the spray-drying process [28]. To verify these assumptions, assays were performed to obtain the number of viable cells in the microcapsules after the encapsulation procedure.

3.2. Cell release and growth on solid medium

In addition to efficiently producing the microcapsules, the ability of *Bacillus* to survive the conditions of the encapsulation process should be assessed. The three microcapsules produced (MC-8, MC-18, and MC-38) maintained survival rates of 7.12 ± 0.04, 7.30 ± 0.04, and 7.21 ± 0.06 log CFU g^{-1}, respectively, immediately after drying. Given that 9 log CFU g^{-1} of *Bacillus* cells/spores were provided before the spray-drying procedure, these values correspond to approximately 80 % of encapsulation efficiency in the resulting powders. This high survival rate can be attributed to the choice of wall materials and the appropriate operating conditions employed.

Previous studies have also demonstrated the protective capacity of starch hydrolysate matrices for encapsulated microorganisms. Chi et al. [16] encapsulated *Bacillus megaterium* NCT-2 using maltodextrin (a starch hydrolysate with DE < 20), achieving about 70 % cell survival after the spray-drying procedure. Similarly, Ma et al. [17] reported around 90 % cell survival when encapsulating *B. sutilis* B99–2 using maltodextrin. Given the effectiveness of starch hydrolysates in microbial encapsulation by spray-drying, the primary goal of this study was to explore the relationship between the properties of the produced microcapsules and the different DE values of the starch hydrolysates, with the aim of applying them as biofertilizers in agriculture.

The ability of encapsulated *Bacillus subtilis* to be released from the microcapsules and grow effectively is a critical factor in determining their efficacy as biofertilizers. Fig. 3 presents the results of cell growth and release behavior in a solid medium (TSA plates), providing insights into how different dextrose equivalent (DE) values influence controlled release properties and bacterial proliferation.

Fig. 3A shows the growth halos formed over time by free and encapsulated bacteria under different conditions. The corresponding quantitative halo measurements in Fig. 3B demonstrate how bacterial growth evolved over 6, 24, and 48 h. At 6 h, the free bacteria (B) and free bacteria mixed with starch hydrolysates (B + DE-8, B + DE-18, B + DE-38) exhibited significant growth halos, while all microcapsule treatments (MC-8, MC-18, MC-38) displayed no visible growth. This indicates that encapsulated bacteria remained entrapped within the microcapsules, delaying their release into the environment. The absence of early growth in encapsulated treatments suggests that the spray-drying encapsulation method effectively retained bacterial cells within the microcapsule structure, preventing immediate diffusion into the medium.

By 24 h, the microcapsule-treated samples (MC-8, MC-18, MC-38) began to show growth halos, confirming that the bacterial cells were gradually released from the microcapsules. At 48 h, all microcapsule treatments reached similar halo sizes to those of free bacteria, indicating that bacterial cells had been fully released and were actively proliferating.

The delayed growth observed for encapsulated bacteria, confirms that microencapsulation modulates bacterial release, preventing an immediate burst release and instead enabling a gradual diffusion of cells into the environment. This property is highly beneficial for biofertilizer applications, as it could lead to extended microbial activity in soil, reducing the need for frequent applications. The findings suggest that all the starch hydrolysates with different DE values studied for microcapsule production are promising candidates for this application. However, the main goal of this study was to further investigate the impact of different DE values in wall materials on the biological properties of the microcapsules. To this end, we evaluated and compared the growth and release profiles of the microcapsules in a wet environment, which will be discussed below.

3.3. Cell release and growth in a wet environment

The release profile and growth behavior of *Bacillus subtilis* in a wet environment provide critical insights into the controlled release properties of the encapsulated bacteria, which are essential for biofertilizer applications. Fig. 4 presents the bacterial viability (log CFU g^{-1}) over time for MC-8, MC-18, and MC-38 microcapsules, demonstrating distinct release profiles that correlate with the dextrose equivalent (DE) value of the starch hydrolysates used as encapsulating agents.

Based on the viability values observed after encapsulation (approximately 7 log CFU g^{-1}), it can be inferred that cell release from the microcapsules occurred within the first 6 h. After this period, the observed values exceeded those recorded for post-encapsulation

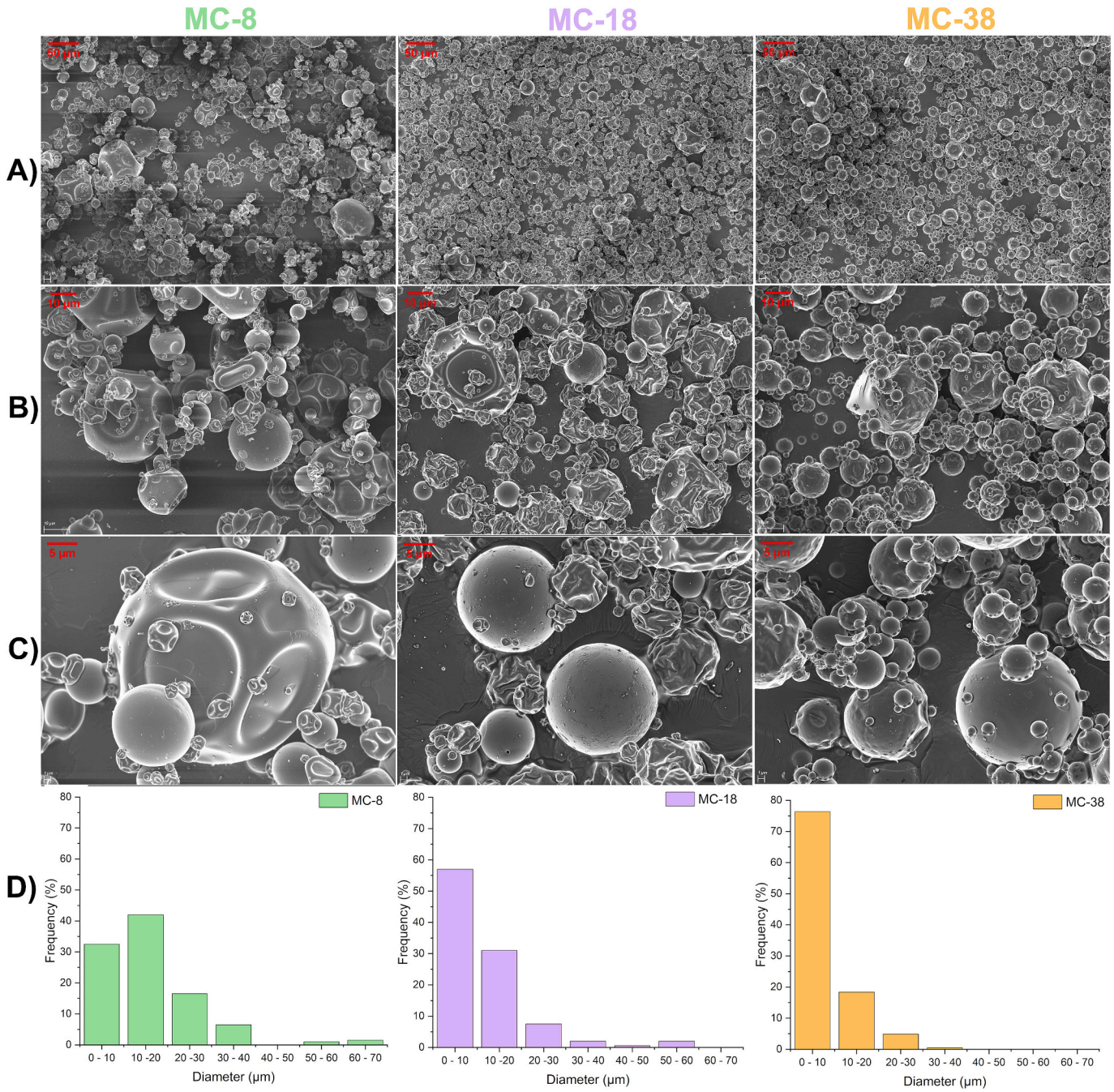

Fig. 2. A) SEM images of the microcapsules produced by spray-drying using different starch hydrolysates as wall materials (DE-8, DE-18 and DE-38) at the magnifications of A) 500 x, B) 2000 x and C) 5000 x. D) Histograms of the particles size distributions.

viability, suggesting potential cell growth due to the consumption of the starch hydrolysates constituting the microcapsules. It is important to note that in this assay, the release of bacterial cells was faster than in the petri dish assay, as the environment was wet.

In terms of cell release, during the initial 6 h, MC-8 exhibited a slower release profile compared to MC-18 and MC-38. This difference may be attributed to the varying DE values of the starch hydrolysates used as wall materials. Higher DE values are associated with more soluble short-chain saccharides [29], which lead to the formation of microcapsules with higher hygroscopicity. This increased water uptake for MC-18 and MC-38 can more easily disrupt its structure, causing the core material to leak [18]. This characteristic may explain the faster release of cells observed in MC-18 and MC-38, while MC-8 maintained a more controlled release profile.

The release profiles can also be linked to the mobility of the polymer chains. Larger polymer chains (from materials with lower DE values) have limited movement [29], which helps regulate the release of encapsulated materials. After 24 h, the viable cell count in MC-18 and MC-38 stabilized. In contrast, MC-8 stabilized only after 48 h, further indicating the slower release of encapsulated cells and, consequently, the slower consumption of the starch hydrolysates. This can be explained by the fact that simpler molecular structures (higher DE materials) allow microbial cells to access and consume sugars more quickly.

These findings highlight the importance of selecting the appropriate encapsulation material based on DE value to optimize biofertilizer performance. By tailoring starch hydrolysate selection, biofertilizer formulations can be designed to meet specific agricultural needs as the ideal release profile will depend on soil conditions and crop

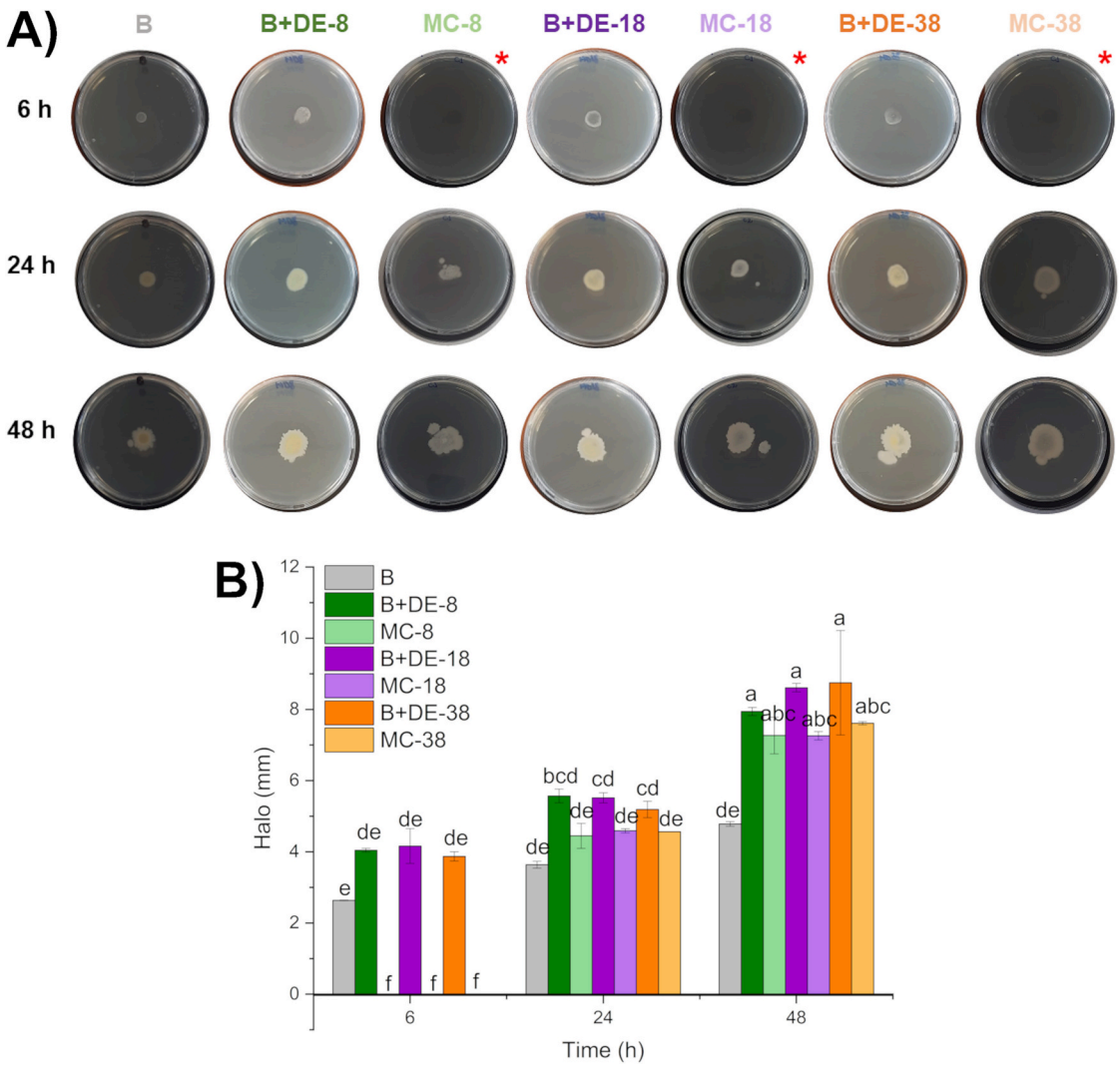

Fig. 3. A) Images of halos representing the growth of the free bacteria (B), the free bacteria in contact with the different DE starch hydrolysates (B + DE-8, B + DE-18 and B + DE-38), and the microcapsules (MC-8, MC-18 and MC-38) in TSA plates at different times (6, 24 and 48 h). B) Graphic constructed with the size of such halos (mm) over time, measured with ImageJ.

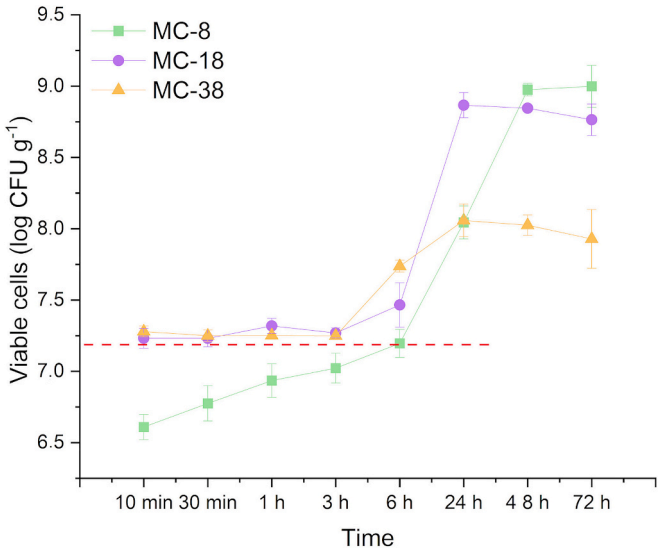

Fig. 4. Cell growth and release profiles in wet environment for the microcapsules (MC-8, MC-18 and MC-38), in terms of log CFU g^{-1}.

requirements. The rapid release could be advantageous in soils requiring immediate bacterial colonization, such as nutrient-deficient fields or hydroponic systems. On the other hand, a slower release may be better suited for long-term field applications, ensuring that bacteria remain active over an extended period without the need for frequent reapplication.

3.4. Maintenance of cell viability under stress conditions

The ability of *Bacillus subtilis* to survive under adverse environmental conditions is a critical factor in determining the effectiveness of microbial biofertilizers. Fig. 5 presents the viability of free and encapsulated bacteria under various stress conditions, including high salinity, acidic pH, heat, and UV exposure. These results provide insights into how encapsulation with starch hydrolysates of different dextrose equivalent (DE) values influences cell protection and stress resistance, which are essential for ensuring bacterial survival in agricultural applications.

Salt stress is a major concern in agriculture, particularly in arid and semi-arid regions, where soil salinization reduces plant growth and microbial survival. Fig. 5A shows the bacterial viability over time in a 50 % (*w/v*) NaCl solution. The free bacteria lost over 10 % of their initial cell count within the first 30 min and continued to decline after 24 h. In contrast, all encapsulated bacteria demonstrated significantly improved

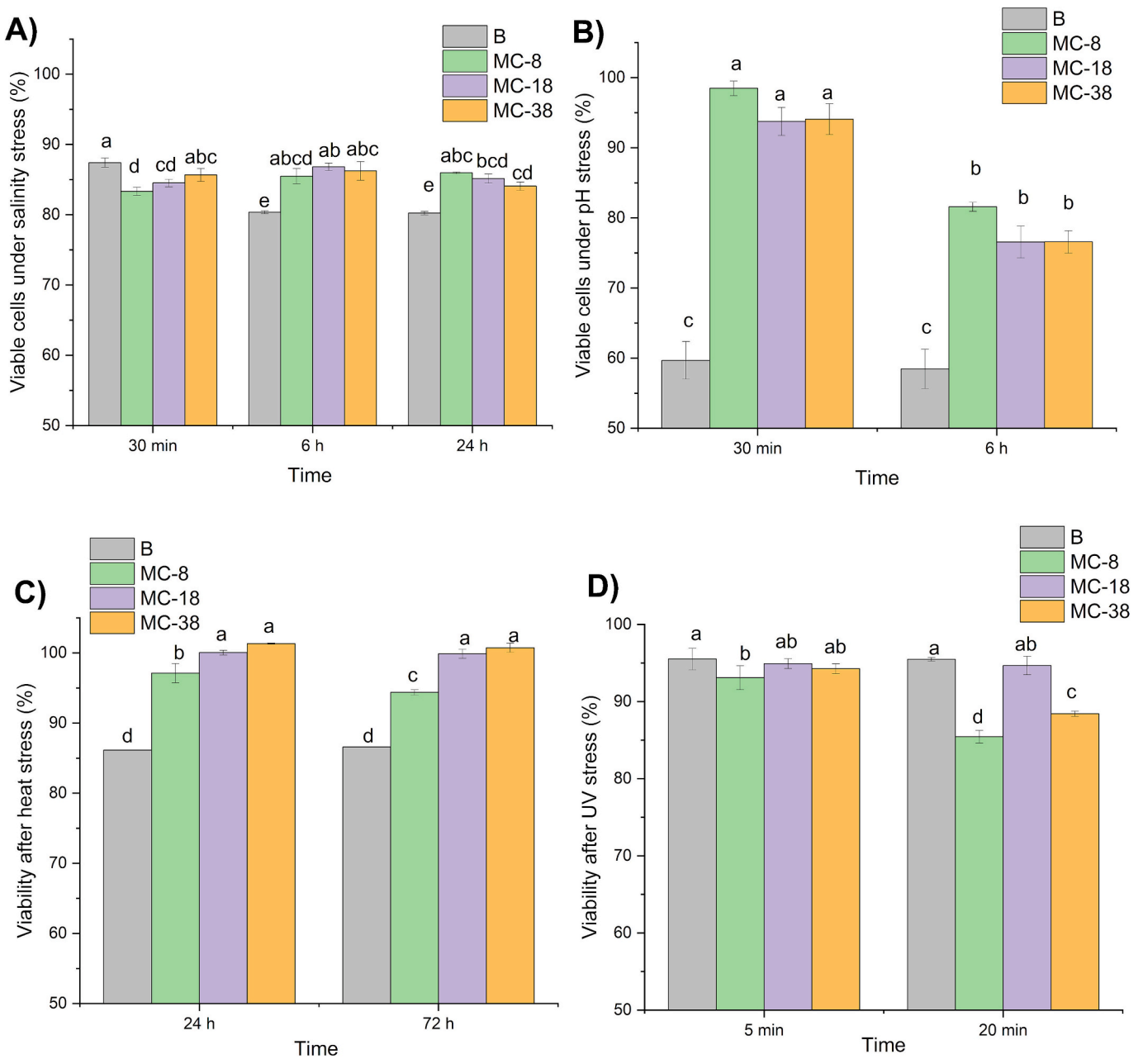

Fig. 5. Percentage of viable cells (%) during A) high salinity and B) acid pH stresses, at different times. Percentage of viable cells (%) after C) heat and D) UV exposure for different periods. The values of 100 % were the viability of the free bacteria and the values of viability after encapsulation obtained for each microcapsule.

resistance to salinity stress over time. The superior performance of the encapsulated cells can be attributed to the protection provided by the matrix, which limits direct exposure to the high-salinity environment, allowing for a more controlled release and gradual adaptation of bacterial cells [18,29]. These findings align with previous studies demonstrating that microencapsulation protects beneficial bacteria from osmotic stress by creating a physical barrier that reduces direct contact with high-salt environments [16].

Acidic soils can also negatively impact microbial activity, as increased H^+ concentrations enhance the mobility and solubility of toxic metals while reducing the availability of nutrients. Therefore, in acidic soils, biofertilizer cells may lose viability, preventing them from fulfilling these beneficial biological functions [30]. Fig. 5B illustrates the protective effect of encapsulation against acidic pH stress. While free bacteria lost approximately 40 % of their viability within the first 30 min, the number of viable cells in the microcapsules remained nearly 95 % in all cases. A similar trend was observed at the 6-h mark, where encapsulated cells consistently exhibited higher viability compared to free bacteria.

Similarly to the protection provided for salinity stress, the key factor contributing to the superior performance of microcapsules is probably related to the physical barrier provided by the wall materials. This barrier shields the cells from direct exposure to the environment, potentially limiting H^+ diffusion and reducing exposure to harsh conditions. As a result, encapsulated cells maintain their viability and metabolic activity more effectively. This protective effect has been previously reported in studies evaluating cell survival under acidic pH conditions, a common assay used in the development of encapsulated probiotic cells [31,32].

Another environmental stress that cells may encounter in field

conditions is high temperature, which can lead to a loss of viability primarily due to the denaturation of essential metabolic proteins [33]. Fig. 5C shows that encapsulated cells exhibit better viability after heat exposure compared to free microorganisms. Beyond the protective effect provided by the wall materials, heat may have enhanced the solubility of and mobility of the microcapsules, promoting the release and movement of cells and the dissolution of sugars, which can be more readily consumed by bacteria. Among the microcapsules, those formulated with higher DE starch hydrolysates (MC-38 and MC-18) demonstrated superior performance compared to the lower DE formulation (MC-8). This may be attributed to the higher concentration of low-molecular-weight species in high-DE materials, which serve as readily accessible nutrients for bacterial cells.

UV radiation is also a significant factor that can reduce bacterial cell viability in field conditions by damaging microbial DNA and RNA [20,34]. Therefore, assessing the effectiveness of microcapsules in protecting cells from UV stress is important. As shown in Fig. 5D, no significant differences were observed between free and encapsulated microorganisms after 5 min of UV exposure. However, after 20 min, free cells and MC-18 microcapsules exhibited similar survival rates, while MC-8 and MC-38 microcapsules provided less effective protection against UV stress. This can be related to the fact that encapsulation alone may not always be sufficient to ensure UV protection, as certain materials lack UV-absorbing properties. When such materials are used as wall components, they may allow radiation to penetrate and compromise cell viability. These findings are consistent with reports stating that microencapsulation offers some protection against UV stress, but complete resistance requires additional shielding components, such as pigments or UV-absorbing agents [35]. The superior UV protection of MC-18 compared to the other microcapsules may be attributed to its intermediate structure, which provides a balance between structural integrity and surface flexibility, reducing light penetration while preventing excessive shrinkage or cracking [34].

3.5. Cell viability during storage of the microcapsules

To assess storage viability, the survival of free and encapsulated bacteria was evaluated at different storage temperatures using the Accelerated Shelf-Life Test (ASLT) method. ASLT is a time-efficient approach that employs mathematical models to predict cell viability during storage. This method is widely used in the food industry for microbial product shelf-life estimation and has been adapted for agricultural applications [22].

Table 1 presents the ASLT-based predictions for the time required for complete viability loss, as well as for reductions to 5, 6, and 7 log CFU. Encapsulated cells demonstrated significantly better stability at all temperatures compared to free cells. The only exception was the time required for MC-8 microcapsules to reach 7 log CFU. Despite this singular case, encapsulation clearly played a protective role in maintaining cell viability during storage.

A direct relationship was observed between storage temperature and viability loss, with higher temperatures accelerating cell degradation in both free bacteria and MC-8 microcapsules. MC-18 and MC-38 followed a similar trend, although their viability at 30 °C and 50 °C remained comparable. The superior performance of MC-18 and MC-38 suggests that higher DE encapsulating matrices provide better microbial protection at moderate and high temperatures. The flexible and hygroscopic nature of high-DE starch hydrolysates may help prevent cell desiccation, reducing oxidative stress and metabolic degradation [34].

It is important to note that increasing initial cell concentrations pre-encapsulation could improve viability. Despite starting with lower counts (~7 log CFU g^{-1}), encapsulated cells outperformed free cells in long-term stability. At 4 °C, MC-8 and MC-18 maintained viability above 6 log CFU g^{-1} for over two years, while MC-38 lasted about a year. At 30 °C, MC-8 and MC-18 lasted ~18 months, with MC-38 stable for about a year. At 50 °C, MC-8 declined faster (8 months), while MC-18 remained stable for ~18 months, and MC-38 maintained viability for ~12 months. Free cells lost viability significantly faster, reaching 8 log CFU within 1.5 months at 4 °C, one month at 30 °C, and 10 days at 50 °C (Supplementary Material 1).

For a two-order viability loss (down to 5 log CFU g^{-1}), MC-8 lasted 55, 33, and 16 months at 4, 30, and 50 °C, respectively. MC-18 remained viable for 46 months at 4 °C and 32 months at higher temperatures. MC-38 showed consistent performance (~23–25 months across all temperatures). In contrast, free cells dropped to 7 log CFU in 3, 2, and < 1 month at 4, 30, and 50 °C, respectively (Table 1).

It should be noted that such ASLT study is a theoretical and mathematical model that aims to predict the behavior of cells viability during storage. Therefore, it should be validated for each case with real-time storage data as it may be influenced by the type of microorganism, the encapsulating material used, humidity levels, among other factors. Thus, these types of studies are important to advance in this research field, to obtain deeper knowledge on the topic and get closer to achieve a consistent methodology to facilitate shelf-life testing. This will allow to obtain accurate results of cell viability during storage using a less time consuming approach.

Overall, our findings emphasize the crucial role of encapsulation by spray-drying in preserving bacterial viability during storage, with the DE value significantly impacting protection efficiency. MC-38 exhibited the highest stability across all temperature levels, making it the most adaptable option for the development of biofertilizers. Hence, these results highlight the need to tailor encapsulation materials to optimize biofertilizer performance, ensuring exstended shelf-life, enhanced microbial survival, and greater agricultural sustainability.

4. Conclusions

This study demonstrated the effective encapsulation of *Bacillus subtilis* via spray-drying using starch hydrolysates with varying dextrose equivalent values as wall materials. Microcapsule morphology was influenced by DE values, with higher DE materials producing smoother, smaller, and more homogeneous particles. Encapsulation efficiency remained consistent across all formulations (~80 %), highlighting the protective role of starch hydrolysates. Controlled release properties were notably enhanced, with MC-8 exhibiting the most gradual release due to its lower solubility. All microcapsules provided significant protection against salinity and acidic pH stresses. Under heat exposure, MC-18 and MC-38 supported higher bacterial survival, likely due to the

Table 1
Results of the ASLT (Accelerated Shelf-Life Test) calculation regarding the time (in months) that would take for the products (free bacteria, MC-8, MC-18 and MC3–8 microcapsules) to loss viabilities (total loss of viability, and until 5, 6 and 7 log CFU) at different temperature values of storage (4, 30 and 50 °C). Initial values of viability were: 9.24 log CFU ml^{-1} for free cells (B), 7.05, 7.30 and 7.29 log CFU ml^{-1} for MC-8, MC-18 and MC-38, respectively.

Temperature	4 °C				30 °C				50 °C			
Products	B	MC-8	MC-18	MC-38	B	MC-8	MC-18	MC-38	B	MC-8	MC-18	MC-38
Months until total loss of viability	12	188	147	73	7	113	105	77	2	54	102	81
Months until reaching 5 log CFU	5	55	46	23	3	33	32	24	1	16	32	25
Months until reaching 6 log CFU	4	28	26	13	2	17	19	14	<1	8	18	14
Months until reaching 7 log CFU	3	1	6	3	2	<1	4	3	<1	<1	4	3

greater molecular mobility of their wall materials. UV resistance was most effective in MC-18, attributed to its intermediate structural properties. Storage stability assessments revealed superior viability of encapsulated cells compared to free bacteria, with higher DE microcapsules demonstrating better resilience to elevated temperatures. These findings provide valuable insights into the role of DE-modified starches in biofertilizer formulation, supporting their potential application as a sustainable strategy to enhance microbial stability and agricultural productivity.

CRediT authorship contribution statement

Marina Momesso Lopes: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Cristiane Sanchez Farinas:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Manuel Martínez Bueno:** Writing – review & editing, Resources, Methodology, Conceptualization. **Pedro J. García-Moreno:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Emilia M. Guadix:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors acknowledge funding from Ministerio de Ciencia e Innovación (PID2023-146901OB-I00). The authors also gratefully acknowledge the financial support provided by the São Paulo State Research Foundation (FAPESP, grant #2024/09631-8), the Brazilian National Council for Scientific and Technological Development (CNPqgrants 441573/2023-1, 402713/2023-0 and 406925/2022-4-INCT Circularity in Polymer Materials), the Coordination for the Improvement of Higher Education Personnel (CAPES, Finance Code 001), the Agronano Network, and the Brazilian Agricultural Research Corporation (Embrapa).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2025.146082.

References

[1] M. Ali, J. Cybulska, M. Frąc, A. Zdunek, Application of polysaccharides for the encapsulation of beneficial microorganisms for agricultural purposes: a review, Int. J. Biol. Macromol. 244 (January) (2023), https://doi.org/10.1016/j.ijbiomac.2023.125366.
[2] P.K. Rai, A. Rai, N.K. Sharma, T. Singh, Y. Kumar, Limitations of biofertilizers and their revitalization through nanotechnology, J. Clean. Prod. 418 (2023) 138194, https://doi.org/10.1016/J.JCLEPRO.2023.138194.
[3] T. Mahanty, S. Bhattacharjee, M. Goswami, P. Bhattacharyya, B. Das, A. Ghosh, P. Tribedi, Biofertilizers: a potential approach for sustainable agriculture development, Environ. Sci. Pollut. Res. 24 (4) (2017) 3315–3335, https://doi.org/10.1007/s11356-016-8104-0.
[4] S. Nosheen, I. Ajmal, Y. Song, Microbes as biofertilizers, a potential approach for sustainable crop production, Sustainability (Switzerland) 13 (4) (2021) 1–20, https://doi.org/10.3390/su13041868.
[5] B.R. Glick, 2012, Mechanisms and Applications, Plant Growth-Promoting Bacteria, 2012.
[6] J. Li, J. Wang, H. Liu, C.A. Macdonald, B.K. Singh, Application of microbial inoculants significantly enhances crop productivity: a meta-analysis of studies from 2010 to 2020. *Journal of sustainable*, Agric. Environ. 1 (3) (2022), https://doi.org/10.1002/sae2.12028.
[7] M.S. Santos, M.A. Nogueira, M. Hungria, Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture, AMB Express 9 (1) (2019), https://doi.org/10.1186/s13568-019-0932-0.
[8] E. Malusà, F. Pinzari, L. Canfora, Efficacy of biofertilizers: challenges to improve crop production, in: In microbial inoculants in sustainable agricultural productivity 2, 2016, https://doi.org/10.1007/978-81-322-2644-4_2 functional applications.
[9] A. Balla, A. Silini, H. Cherif-Silini, A. ChenariBouket, F.N. Alenezi, L. Belbahri, Recent advances in encapsulation techniques of plant growth-promoting microorganisms and their prospects in the sustainable agriculture, Applied sciences 2022 12 (2022), https://doi.org/10.3390/APP12189020 page 9020, 12(18), 9020.
[10] M. Brondi, C. Florencio, L. Mattoso, C. Ribeiro, C. Farinas, Encapsulation of Trichoderma harzianum with nanocellulose/carboxymethyl cellulose nanocomposite, Carbohydr. Polym. 295 (2022) 119876, https://doi.org/10.1016/j.carbpol.2022.119876.
[11] J.L. Oliveira, L.F. Fraceto, A. Bravo, R.A. Polanczyk, Encapsulation strategies for Bacillus thuringiensis: from now to the future, J. Agric. Food Chem. 69 (16) (2021) 4564–4577, https://doi.org/10.1021/acs.jafc.0c07118.
[12] C. Berraquero-García, R. Pérez-Gálvez, F.J. Espejo-Carpio, A. Guadix, E.M. Guadix, P.J. García-Moreno, Encapsulation of bioactive peptides by spray-drying and electrospraying, Foods 12 (10) (2023) 2005, https://doi.org/10.3390/foods12102005.
[13] C.I. Piñón-Balderrama, C. Leyva-Porras, Y. Terán-Figueroa, V. Espinosa-Solís, C. Álvarez-Salas, M.Z. Saavedra-Leos, Encapsulation of active ingredients in food industry by spray-drying and Nano spray-drying technologies, Processes 8 (8) (2020) 889, https://doi.org/10.3390/pr8080889.
[14] N.E. Rahmani-Manglano, M. Tirado-Delgado, P.J. García-Moreno, A. Guadix, E. M. Guadix, Influence of emulsifier type and encapsulating agent on the in vitro digestion of fish oil-loaded microcapsules produced by spray-drying, Food Chem. 392 (2022) 133257, https://doi.org/10.1016/j.foodchem.2022.133257. ISSN 0308-8146.
[15] M. Schoebitz, M.D.L. Belchí, Encapsulation Techniques for Plant Growth-Promoting Rhizobacteria, For Sustainable Agriculture, In Bioformulations, 2016, https://doi.org/10.1007/978-81-322-2779-3_14.
[16] Y. Chi, D. Wang, M. Jiang, S. Chu, B. Wang, Y. Zhi, P. Zhou, D. Zhang, Microencapsulation of Bacillus megaterium NCT-2 and its effect on remediation of secondary salinization soil, J. Microencapsul. 37 (2) (2020), https://doi.org/10.1080/02652048.2019.1705409.
[17] X. Ma, X. Wang, J. Cheng, X. Nie, X. Yu, Y. Zhao, W. Wang, Microencapsulation of Bacillus subtilis B99-2 and its biocontrol efficiency against Rhizoctonia solani in tomato, Biol. Control 90 (2015), https://doi.org/10.1016/j.biocontrol.2015.05.013.
[18] Z. Xiao, J. Xia, Q. Zhao, Y. Niu, D. Zhao, Maltodextrin as wall material for microcapsules: a review, Carbohydr. Polym. 298 (2022) 120113, https://doi.org/10.1016/J.CARBPOL.2022.120113.
[19] Y. Bashan, de-Bashan, L. E., Prabhu, S. R., & Hernandez, J. P., Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998-2013), Plant Soil 378 (1–2) (2014) 1–33, https://doi.org/10.1007/s11104-013-1956-x.
[20] R.P. John, R.D. Tyagi, S.K. Brar, R.Y. Surampalli, D. Prévost, Bio-encapsulation of microbial cells for targeted agricultural delivery, Crit. Rev. Biotechnol. 31 (3) (2011) 211–226, https://doi.org/10.3109/07388551.2010.513327.
[21] I.S. Chronakis, On the molecular characteristics, compositional properties, and structural-functional mechanisms of maltodextrins: a review, Crit. Rev. Food Sci. Nutr. 38 (7) (1998), https://doi.org/10.1080/10408699891274327.
[22] M.M. Lopes, L.A. Lodi, C.A. Oliveira-Paiva, C.S. de Farinas, Emulsion/cross-linking encapsulation of Bacillus in starch/PVA-based microparticles for agricultural applications, ACS Agricultural Science and Technology. (2024), https://doi.org/10.1021/acsagscitech.4c00029.
[23] S.E. Azaroual, N. El Mernissi, Y. Zeroual, B. Bouizgarne, I. Meftah Kadmiri, Effect of Bacillus spp. strains on wheat nutrient assimilation and bioformulation by new spray drying approach using natural phosphate powder, Dry. Technol. 40 (13) (2022) 2630–2644, https://doi.org/10.1080/07373937.2021.1950170.
[24] I. Siemons, R.G.A. Politiek, R.M. Boom, R.G.M. van der Sman, M.A.I. Schutyser, Dextrose equivalence of maltodextrins determines particle morphology development during single sessile droplet drying, Food Res. Int. 131 (2020), https://doi.org/10.1016/j.foodres.2020.108988.
[25] A.A. Ghani, S. Adachi, H. Shiga, T.L. Neoh, S. Adachi, H. Yoshii, Effect of different dextrose equivalents of maltodextrin on oxidation stability in encapsulated fish oil by spray drying, Biosci. Biotechnol. Biochem. 81 (4) (2017), https://doi.org/10.1080/09168451.2017.1281721.
[26] Y. Ma, C.J. Hou, H.X. Wu, H.B. Fa, J.J. Li, C.H. Shen, D. Li, D.Q. Huo, Synthesis of maltodextrin-grafted-cinnamic acid and evaluation on its ability to stabilize anthocyanins via microencapsulation, J. Microencapsul. 33 (6) (2016), https://doi.org/10.1080/02652048.2016.1223201.
[27] L. Herrmann, D. Lesueur, Challenges of formulation and quality of biofertilizers for successful inoculation, Appl. Microbiol. Biotechnol. 97 (20) (2013) 8859–8873, https://doi.org/10.1007/s00253-013-5228-8.
[28] M.Z. Saavedra-Leos, M. Román-Aguirre, A. Toxqui-Terán, V. Espinosa-Solís, A. Franco-Vega, C. Leyva-Porras, Blends of carbohydrate polymers for the co-microencapsulation of Bacillus clausii and quercetin as active ingredients of a functional food, Polymers 2022 14 (2022), https://doi.org/10.3390/POLYM14020236 page 236, 14(2), 236.
[29] Z. Saavedra-Leos, C. Leyva-Porras, S.B. Araujo-Díaz, A. Toxqui-Terán, A.J. Borrás-Enríquez, Technological application of maltodextrins according to the degree of polymerization, Molecules 20 (12) (2015), https://doi.org/10.3390/molecules201219746.

[30] N. Chowdhury, D.J. Hazarika, G. Goswami, U. Sarmah, S. Borah, R.C. Boro, M. Barooah, Acid tolerant bacterium *Bacillus amyloliquefaciens* MBNC retains biocontrol efficiency against fungal phytopathogens in low pH, Arch. Microbiol. 204 (2022) 124, https://doi.org/10.1007/s00203-021-02741-5.

[31] C. Ji, Y. Gao, X. Huang, F. Yan, T. Yu, Microencapsulation of Bacillus smithii XY1 by spray drying and evaluation for treatment of inflammatory bowel disease, Food Biosci. 53 (2023), https://doi.org/10.1016/j.fbio.2023.102542.

[32] M. Li, S. Suwanangul, P. Jaichakan, N. Narkprasom, S. Kraithong, K. Narkprasom, P. Sangsawad, viability, bioactivity, and in vitro-simulated gastrointestinal digestion. Foods 2023, Vol. 12, page 3692, 12(19), 3692, in: Innovative insights for establishing a Synbiotic relationship with Bacillus coagulans, 2023, https://doi.org/10.3390/FOODS12193692.

[33] W.H. Coleman, D. Chen, Y.Q. Li, A.E. Cowan, P. Setlow, How moist heat kills spores of Bacillus subtilis, J. Bacteriol. 189 (23) (2007), https://doi.org/10.1128/JB.01242-07.

[34] R. Hu, D. Dong, J. Hu, H. Liu, Improved viability of probiotics encapsulated in soybean protein isolate matrix microcapsules by coacervation and cross-linking modification, Food Hydrocoll. 138 (2023), https://doi.org/10.1016/j.foodhyd.2023.108457.

[35] M.M. Lopes, C.A. Oliveira-Paiva, C.S. De Farinas, Modification of pectin/starch-based beads with additives to improve Bacillus subtilis encapsulation for agricultural applications, Int. J. Biol. Macromol. 246 (2023), https://doi.org/10.1016/j.ijbiomac.2023.125646.