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# Impact of drying methods and storage conditions on the reactivation of *Sporosarcina pasteurii* for microbial induced carbonate precipitation

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The drying of bacteria using various methods is a widely used technique for long-term stabilization across different applications. For organisms capable of producing the enzyme urease, which are used in microbial induced carbonate precipitation (MICP), drying may also offer promising new fields for application. In the present study, two drying methods, fluidized bed drying and freeze-drying, were applied to *Sporosarcina pasteurii*, both with and without the commonly used cryoprotectant maltodextrin. The dried samples were evaluated in terms of cell viability, storage stability (based on urease activity) at three different temperatures (room temperature, 4 °C and −20 °C), and their subsequent performance after 92 days of storage for a typical MICP application, aiming to increase the uniaxial compressive strength of quartz sand columns. Maltodextrin positively affected cell viability and urease enzyme stability, with the freeze-dried powder showing the highest cell viability at 21%, while fluidized bed drying resulted in less than 1% viability. Storage temperature influenced urease stability, with a decrease in enzyme activity at −20 °C being 22.63%, and showed a further decrease at higher temperatures, with 67.86% at room temperature and 64.23% at 4 °C, respectively for the freeze-dried powders. Nevertheless, both powders from the two drying methods improved the compressive strength of sand columns via MICP, with UCS values reaching up to 10.81 $\frac{N}{mm^2}$ for the freeze-dried powders. The findings demonstrate that both fluidized bed and freeze-drying techniques allow *Sporosarcina pasteurii* to be stored at room temperature without the need for a protective agent, highlighting their practicality for MICP applications and demonstrating their potential for large-scale use in civil engineering and geoengineering.

KEYWORDS

***Sporosarcina pasteurii*, microbial induced carbonate precipitation (MICP), fluidized bed drying, lyophilization (freeze-drying), storage, cell viability, protectant**

# 1 Introduction

The formation of calcium carbonate precipitation caused by various microorganisms is well researched and commonly referred to as microbial induced carbonate precipitation (MICP). These microorganisms are capable of precipitating calcium carbonate ($CaCO_3$), such as bacterial Baidya et al. (2023) or fungal Devgon et al. (2024) organisms. However, the bacterium *Sporosarcina pasteurii* is commonly preferred for the biocementation process, due to its outstanding ability to produce large amounts and high-quality $CaCO_3$, form spores under certain conditions and remain usable over a wide pH range for MICP Naveed et al. (2020); Khoshtinat (2023). Since various microorganisms can be used for MICP, there are also multiple metabolic pathways that can be utilized for this process, including ureolytic processes, photosynthesis, denitrification, or sulfate reduction Zhu and Dittrich (2016). Each pathway contributes uniquely to the biomineralization process, facilitating the deposition of $CaCO_3$ under diverse environmental conditions. These $CaCO_3$ precipitations are used for different applications, such as crack healing in self healing concrete Khushnood et al. (2020), dust control Zhou et al. (2023), soil stabilization Mujah et al. (2017) or immobilization of heavy metals Wang et al. (2023).

When working with MICP, the largest portion of current research and applications utilize liquid cultures that are cultivated immediately before their use. The storage of liquid cultures of *S. pasteurii* under different conditions (25 °C, 4 °C, and −18 °C) over 17 or 40 days showed a significant decline in cell viability and urease activity over the storage period Erdmann et al. (2022); Mehring et al. (2021). This means that liquid cultures have a limited storage stability and new liquid cultures have to be prepared each time MICP is used.

As an alternative to the liquid cultures, dried bacteria or enzymes can also be used for MICP applications. Dried bacteria can offer many advantages compared to liquid cultures, such as longer storage stability, require less storage or transport volume, and can also be stored at moderate temperatures Peiren et al. (2015). There are already several drying methods for microorganisms that are well established in other areas of application. The most important and widely known drying techniques for bacteria include freeze-drying (lyophilization), spray drying, fluidized bed drying, and vacuum drying Broeckx et al. (2016); Tan et al. (2018). Lactic acid bacteria are frequently dried due to their importance in the food industry, making them the primary focus of research on microorganism drying Morgan et al. (2006); Santivarangkna et al. (2007). During the process of freeze-drying, cells are first frozen and then dehydrated by sublimating the frozen moisture under high vacuum conditions. The process of fluidized bed drying often requires a carrier material, such as microcrystalline cellulose (MCC), which has been demonstrated to support the drying of different organisms Son et al. (2022). In general, the drying process removes physically bound and unbound water from cells. Furthermore, physiological reactions and the metabolism are inhibited or inactivated, respectively. For instance, different bacteria, such as *Enterococcus faecium* M74 Stummer et al. (2012), *Lactobacillus plantarum* Bensch et al. (2014); Strasser et al. (2009), *Lactobacillus reuteri* Schell and Beermann (2014), *Lactobacillus lactis 1464* Wirunpan et al. (2016), and *E. faecium* Strasser et al. (2009), have been successfully dried using fluidized bed drying.

To achieve a higher cell viability, a lot of different cryoprotectants can be used to protect the cells during the drying process and the storage period. Different types of sugar, such as maltodextrin or sucrose (Oluwatosin et al. (2022); Bensch et al. (2014); Wang et al. (2020), are often used as they enhance desiccation tolerance by stabilizing membranes and proteins, replacing water around polar residues in macromolecular structures Morgan et al. (2006). It is also possible to use (skim) milk powder Ge et al. (2024); Oluwatosin et al. (2022); Wang et al. (2020), as proteins may play a more critical role than sugars in desiccation protection, which could explain the effectiveness of protein-rich substances. Instead of relying on active bacterial cells in liquid, a promising approach involves the application of bacterial spores. For example, *S. pasteurii* was utilized to enhance the thermal stability and flame resistance of cellulose aerogels, containing bacteria and yeast, through calcium carbonate biomineralization Jones and Srubar (2022). Further innovations include the immobilization of microorganisms or spores on various materials to enhance their application. Examples include immobilization on aggregates Khushnood et al. (2020), mixtures of metakaolin and sodium silicate Jadhav et al. (2018), or perlite Jiang et al. (2020); Alazhari et al. (2018), often used in self-healing systems alongside nutrients and protective layers. Clay particles were also used as carriers for immobilized microorganisms, which are used for MICP Wiktor and Jonkers (2011). Additionally, direct mixing of MICP microorganisms with cement has been explored Jonkers et al. (2010), as well as microencapsulation techniques using spray drying, freeze-drying, or extrusion Pungrasmi et al. (2019). These microencapsulation methods help in protecting the microorganisms while facilitating their activation when conditions are favourable Xu et al. (2024); Wang et al. (2014).

It has already been shown that the organism *S. pasteurii* used in the present study can be dried using different methods for various purposes. The organism was freeze-dried for SEM analysis to determine bacterial characteristics (morphology structure such as length and width) Chen et al. (2021); Fu et al. (2022) or for long-term storage and sale of small quantities for strain preservation, allowing the organism to be cultivated in liquid media again Kahani et al. (2020); Li et al. (2023); Omoregie et al. (2020); Røyne et al. (2019). There is also literature in which lyophilised MICP-compatible bacterial cells, including *S. pasteurii*, have been reused for various MICP applications. Terzis and Laloui (2018) and Terzis and Laloui (2019) produced freeze-dried *S. pasteurii* samples and subsequently tested them with varying biomass and urea concentrations to evaluate their functionality for MICP applications in sand with different properties. Similar results were shown by Tuttle et al. (2025), which also used freeze-dried *S. pasteurii* and investigated viability and MICP performance in sand columns and field applications. Valencia-Galindo et al. (2021) compared MICP capable freeze-dried powder with freshly cultivated bacteria, in which the fresh bacteria deliver better results. Furthermore, Fu et al. (2022) prepared microbial healing agents by drying *S. pasteurii* spores at 40 °C.

There is currently a gap in literature regarding the investigation of different drying methods without and with sugar-based protectants and the following use of the cells for MICP applications. Although some studies have explored the drying and reuse of

bacteria for MICP applications, no systematic, direct comparison between freeze-drying and fluidized bed drying has been reported. In particular, their effects on the viability of vegetative *S. pasteurii* cells, the role of sugar-based protectants such as maltodextrin, and their subsequent performance in soil stabilization remain unexamined. Therefore, the research objectives of the present study are to introduce a new approach by directly comparing two of the most commonly used drying methods for microorganisms: freeze-drying, which is widely used for drying microorganisms, and fluidized bed drying, which is considered to be very gentle due to its moderate drying temperatures in comparison to spray drying. The study also evaluates the effects of maltodextrin under these conditions on *S. pasteurii*. In addition, the long-term storage stability of the dried bacteria over 90 days and their subsequent use in soil stabilization will be investigated, an aspect that has not been studied in previous research.

# 2 Materials and methods

## 2.1 Cultivation and preparation of *Sporosarcina pasteurii*

The used bacterium *S. pasteurii* DSM 33 was cultivated in parallel bioreactors (Bioengineering AG, RALF Plus-System) with a working volume of 2.5 L. To collect enough bacterial biomass, three reactors were harvested for the fluidized bed drying and one for the freeze-drying experiments. For the reactors, the $CaSo^{+}$ medium was prepared according to Lapierre et al. (2022) with the following components: 10 g/L glucose, 20 g/L urea, 1.7 g/L $K_2HPO_4$, 15 g/L casein peptone, 5 g/L soy peptone, and 5 g/L NaCl. After preparation, the medium was adjusted to a pH of 7.5 with NaOH. It also contained 50 mL/L of micronutrient stock solution (8.54 g/L $MgCl_2 \cdot 6H_2O$, 0.56 g/L $MnSO_4 \cdot 3H_2O$, 0.18 g/L $ZnSO_4 \cdot 7H_2O$, 0.085 g/L $CoSO_4 \cdot 7H_2O$, 0.08 g/L $CuSO_4 \cdot 5H_2O$, 0.06 g/L $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.2 g/L $NiCl_2 \cdot 6H_2O$, and 0.2 g/L EDTA), and 5 mL/L of iron stock solution (1 g/L $FeCl_3 \cdot 6H_2O$ and 1 g/L $FeCl_2$). The concentrated solutions of urea, trace elements, iron, glucose, and $K_2HPO_4$ were added to the bioreactor after autoclaving to achieve the final concentration. The addition was performed at a medium temperature of 30 °C using a 0.22 $\mu$m PES sterile filter through a septum. For each bioreactor, 20 mL of a 24 h *S. pasteurii* preculture, cultivated in 24 mL of $CaSo^{+}$ medium in a 250 mL shake flask at 30 °C and 250 rpm in a shaking incubator (IKA-Werke GmbH, KS4000i, shaking diameter 20 mm), was used as the inoculum. The preculture was inoculated from a −80 °C cryo culture. All settings were identical for each bioreactor. The temperature was set to 30 °C and pH was only measured, not controlled. The airflow and stirrer speed were regulated through a controlled cascade, depending on the measured $pO_2$ value (20% setpoint). After a cultivation time of 24 h, each bioreactor was harvested, the $OD_{600}$ was measured, and the cells were examined under a microscope for contamination control. Additionally, the urease activity was measured with a conductivity method, according to the method of Whiffin, (2004). Once the cells were removed from the bioreactors, sterile conditions were no longer maintained. For the washing procedure, the cells were centrifuged in 1,300 mL stainless steel beakers at 5,100 rpm (8,578 rcf) for 60 min at 4 °C (Sigma Laborzentrifugen GmbH, Sigma 8K). After this, the supernatant was removed and the cells were resuspended in 9 g/L NaCl. This procedure was repeated one more time. Afterwards, a $OD_{600}$ of 35 was set with 9 g/L NaCl and the cells were stored at 4 °C until the drying experiments were performed.

## 2.2 Fluidized bed drying

For fluidized bed drying, a laboratory fluidized bed processor (DMR Prozesstechnologie GmbH, WFP-Mini) was used. The complete test setup is shown in Figure 1A. The *S. pasteurii* cells produced in accordance with Section 2.1 were sprayed into the reactor chamber using a spray lance (25 mm) with a 0.8 mm nozzle and an air flow of 100% nitrogen. A total of 652 g of bacterial suspension with an $OD_{600}$ of 35 was used, either with or without 115 g maltodextrin (15% (w/w)). The maltodextrin was added directly to the bacterial suspension and stirred until it was completely dissolved. During the drying process, the bacterial culture was stirred continuously to guarantee an identical composition during spraying. The bacterial suspension was transported through silicone tubes (ID = 1.6 mm, OD = 4.8 mm) with the help of a peristaltic pump (Watson-Marlow GmbH, 120S/DV). As carrier for the bacterial cells 300 g of MCC, a carrier often used for drying microorganisms, with a size of 100–200 $\mu$m (Vivapur MCC Spheres 100, kindly provided by J. Rettenmaier and Söhne GmbH + Co. KG, Rosenberg, Germany) was used for each experiment. All device settings and measured parameters from the fluidized bed drying experiments are summarized in Table 1 for the processes carried out with and without protectant.

At the beginning of the experiment, the three filter cartridges (Type KE 2671 Ti07/1–0.13 V4A FRV Ø 75 mm x L 200 mm) of the fluidized bed dryer were examined for adhesions during the drying process. For this, the experiment was briefly stopped, and the fluidized bed reactor was opened. The drying process was completed once the entire bacterial suspension had been transported into the fluidized bed drying reactor. Afterwards, the resulting powder was dried for 30–45 min with the same settings but without the addition of bacterial suspension, hereinafter referred as post-drying. Afterwards, the powder was transferred into 50 mL tubes and used for the upcoming experiments, described in Section 2.4 and 2.5.

## 2.3 Freeze-drying

For the freeze-drying of the bacterial suspension prepared according to Section 2.1, a laboratory system (ZIRBUS technology GmbH, VaCo 2, −80 °C) was used. The experimental setup and settings are illustrated in Figure 1B. The bacterial cells were divided into two portions. To one portion, maltodextrin was added to a final concentration of 15% (w/w) and mixed until fully dissolved. The bacterial suspensions were then dispensed into 50 mL tubes, 30 g for the samples containing maltodextrin and 22.5 g for those without, and frozen at −20 °C for 16 h. The difference in filling weight reflected the different densities of

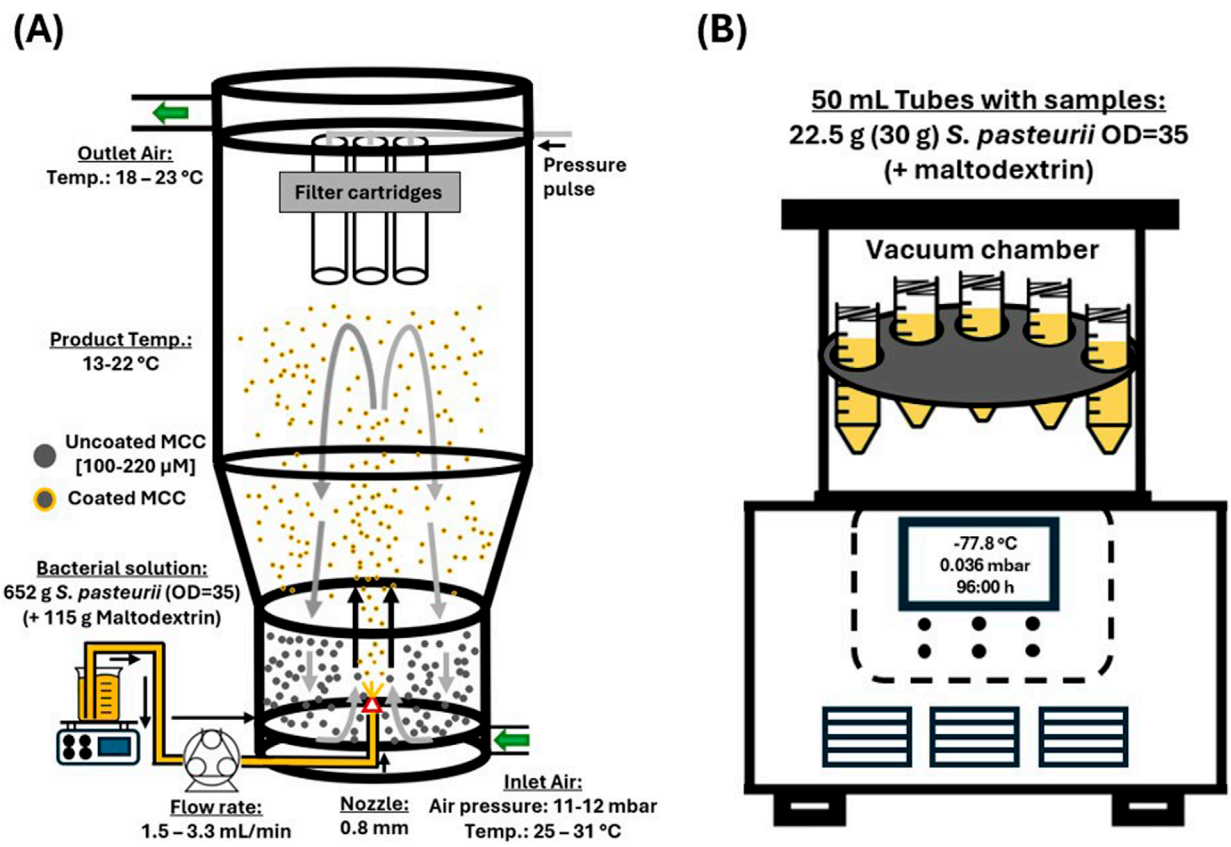


FIGURE 1
Graphical illustration of the two different drying methods for *Sporosarcina pasteurii* used in the present study, fluidized bed drying **(A)** and freeze-drying **(B)**. The illustration for the freeze-drying is based on Taskin (2020).

TABLE 1 Fluidized bed drying settings and measured temperature values.

| Setting | Inlet pressure [mbar] | Temperature [°C] | | | Mass flow rate [g/min] | Time [h] |
|---|---|---|---|---|---|---|
| | | Supply air | Product | Exhaust air | | |
| w/o maltodextrin | 11–12 | 25–31 | 13–20 | 18–22 | 1.5–3.3 | 4 |
| w/ maltodextrin | 11–12 | 28–31 | 16–22 | 21–23 | 2.36 | 6 |

the suspensions, ensuring equal fill heights in the 50 mL tubes. Subsequently, the tubes were placed into the freeze-drier for 96 h at −77.8 °C and 0.036 mbar. The resulting dried cells, without or with maltodextrin, were manually homogenized in the 50 mL tube using a spatula and subsequently used in the experiments described in Sections 2.4 and 2.5.

## 2.4 Imaging and SEM

For the real-time observation of particle imaging during calcium carbonate precipitation, an analytical imaging instrument was used (Technobis Crystallization Systems, Crystalline). Therefore, quartz sand and powder without the maltodextrin was mixed and filled into a 20 mL glass vial. After the vial was placed in the device and the camera focused, cementation solution was filled in. Then the experiment was started at 30 °C and every 10 s an image was taken. After 24 h the experiment was stopped. The microstructural analysis was conducted using a high-resolution scanning electron microscope (SEM) (LYRA3, Tescan). For sample preparation, the specimens were mounted on a sample holder using adhesive carbon tape and then sputter-coated with a thin layer of platinum (approximately 1 nm thick). Imaging was carried out with the secondary electron detector at 5 keV.

## 2.5 Analysis of dried bacteria

To compare the two different drying methods in terms of particle distribution, cell viability after the drying process, and final

application after rehydration for MICP, identical experiments were conducted for both types of resulting powders.

### 2.5.1 Weight, particle distribution and residual moisture

After the drying process, the total weight of each powder was measured directly with a scale (Mettler-Toledo GmbH, XS6002-S DR). Afterwards, about 2 g of the dried powder was used to analyse the particle distribution via laser scattering (HORIBA Europe GmbH, LA-960), and 2.5 g were weighed and heated at 105 °C until the weight remained constant within ±1 mg for 50 s to determine the residual moisture as a weight percent (Mettler-Toledo GmbH, HB43-S).

### 2.5.2 Cell viability

In the present study, the viability of *S. pasteurii* cells was determined, where cell viability refers to their ability to form colony-forming units (CFUs), similar to Stummer et al. (2012); Ge et al. (2024). To analyse the cell viability of *S. pasteurii* after the different drying procedures, 5 g of each powder were transferred in 15 mL tubes and filled up with 9 g/L NaCl solution. To detach the cells from the carrier or to rehydrate them, the tubes were shaken in horizontal position on an incubator shaker for 1 h at 150 rpm (shaking diameter 20 mm). The tubes were stored for 5 min to allow the MCC to settle. Afterwards, 2 mL of the supernatant from the top were decanted and transferred in 2 mL tubes. The cells were washed with 9 g/L NaCl by centrifugation at 9,614 rcf for 30 min (Hermle Labortechnik GmbH, Z233 MK-2) and the cell pellet was resuspended in fresh 9 g/L NaCl solution to remove the maltodextrin and other substances from the bacterial cells. From this suspension, the $OD_{600}$ was measured, and based on the result, the samples were diluted to a final $OD_{600}$ of 1 with 9 g/L NaCl. With this suspension, a dilution series was prepared, and 100 $\mu$L were homogeneously plated on each of three CaSo-Urea agar plates for each dilution range. The CaSo-Urea agar plates consisted of 15 g/L casein peptone, 5 g/L soy peptone, 5 g/L NaCl, 15 g/L agar and the pH was adjusted to 9.3 with NaOH. After autoclaving, the agar was cooled down to 60 °C and a sterile 2 M urea solution was added to reach a final concentration of 20 g/L. After 2 days of incubation at 30 °C in an incubation cabinet (Binder GmbH, BF115), the CFUs were counted. As a result, the dilution series was chosen in a way that the count ranged between 30 and 300 CFUs on each agar plate. A 24 h cultivated *S. pasteurii* culture in $CaSo^{+}$ media was diluted to an $OD_{600}$ of 1, plated identically and used as control sample.

### 2.5.3 Storage

To identify the influence of storage time on the different powders up to 92 days at three different temperatures, the powders were split in three settings. Therefore, the powders were transferred into 50 mL tubes and stored in the laboratory at room temperature, in the refrigerator at 4 °C and in the freezer (−20 °C). All samples were covered from light exposure. Each week, a sample of 2.5 g from the fluidized bed dried bacteria from all three storage conditions, with or without maltodextrin, was analysed at approximately 20 °C for its urease activity, according to Whiffin, (2004). From the freeze-dried bacteria powders, 0.05 g was taken and measured identically. For better comparability, the measured urease values of the different powders were adjusted in relation to the various factors affecting the weight of the powder, such as the weight of the MCC or the protectant. Table 2 provides a more detailed discussion of the specifics.

### 2.5.4 Usage for MICP

After the storage period of 92 days, the different powders were tested for the process of MICP at room temperature in quartz sand columns. Therefore, the quartz sand was mixed with a quantity of powder such that a urease activity of 40 $\frac{\mu S}{cm*s}$ was achieved in the column. For the validation of the urease activity, the last measured value after 92 days was used. After mixing the quartz sand with the required amount of powder, a total mass of 80 g was achieved. The mixture was then filled into two 3D-printed syringe halves made of polylactide material. The mixtures were positioned between 200 mg of glass wool and 5 g of coarse gravel (grain size: 3.5-5 mm) at the inlet, and 20 g of coarse gravel along with 200 mg of glass wool at the outlet. This setup was prepared according to Hanisch et al. (2024). Subsequently, 25 mL of a cementation solution containing 1.5 M urea and 1.1 M $CaCl_2$ was pumped through at a flow rate of 0.25 mL/min for 100 min. After pausing the flow for another 100 min, 25 mL of the cementation solution was pumped through the column once more. This cycle of pumping and pausing was repeated six times in total. Following 24 h of biocementation, the samples were left to rest in the syringe halves for an additional 24 h to allow for drainage. Two control samples were prepared using a 24 h *S. pasteurii* liquid culture, cultivated in the same manner as previously described. One control consisted of pure quartz sand, while the other contained 10 g of MCC and 70 g of quartz sand, to closely replicate the composition of the sand columns prepared with bacterial coated MCC. For both control samples, 25 mL of the *S. pasteurii* culture was prepared to achieve a total urease activity of 40 $\frac{\mu S}{cm*s}$. This suspension was pumped into the sand columns at a flow rate of 0.25 mL/min, followed by the cementation solution, using the same procedure as for the sand columns prepared with the different bacterial powders. All sand columns were subsequently taken out of the molds and stored at 40 °C for a period of 2 weeks. The test specimens were then sawed into three pieces of equal length (25–30 mm). The saw edges were straightened with sandpaper to ensure plane-parallel test surfaces for the compression test. Each column segment was placed in a tabletop testing machine (Zwick GmbH and Co. KG, Z2.5) and tested for uniaxial compressive strength (UCS). To eliminate the influence of quartz sand and MCC strength, negative controls were conducted for MCC and for quartz sand.

## 3 Results

### 3.1 Post-drying sample analysis (residual moisture, image analysis, cell viability)

#### 3.1.1 Fluidized bed drying

After the fluidized bed drying process and the post-drying, the residual moisture of the powders was measured, resulting in

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TABLE 2 Proportions of different components of the dried biomass in different settings.

| Proportions | Freeze-drying | | Fluidized bed drying | |
|---|---|---|---|---|
| | w/o maltodextrin | w/ maltodextrin | w/o maltodextrin | w/ maltodextrin |
| Bacterial cells | 64.41% | 7.91% | 4.20% | 3.09% |
| NaCl | 25.74% | 3.16% | 1.68% | 1.24% |
| Maltodextrin | n.a | 79.69% | n.a | 25.66% |
| Water content (moisture) | 9.85% | 9.24% | 3.10% | 3.08% |
| MCC | n.a | n.a | 91.02% | 66.94% |
| Balancing factor | 1 | 8.14 | 1 | 1.36 |

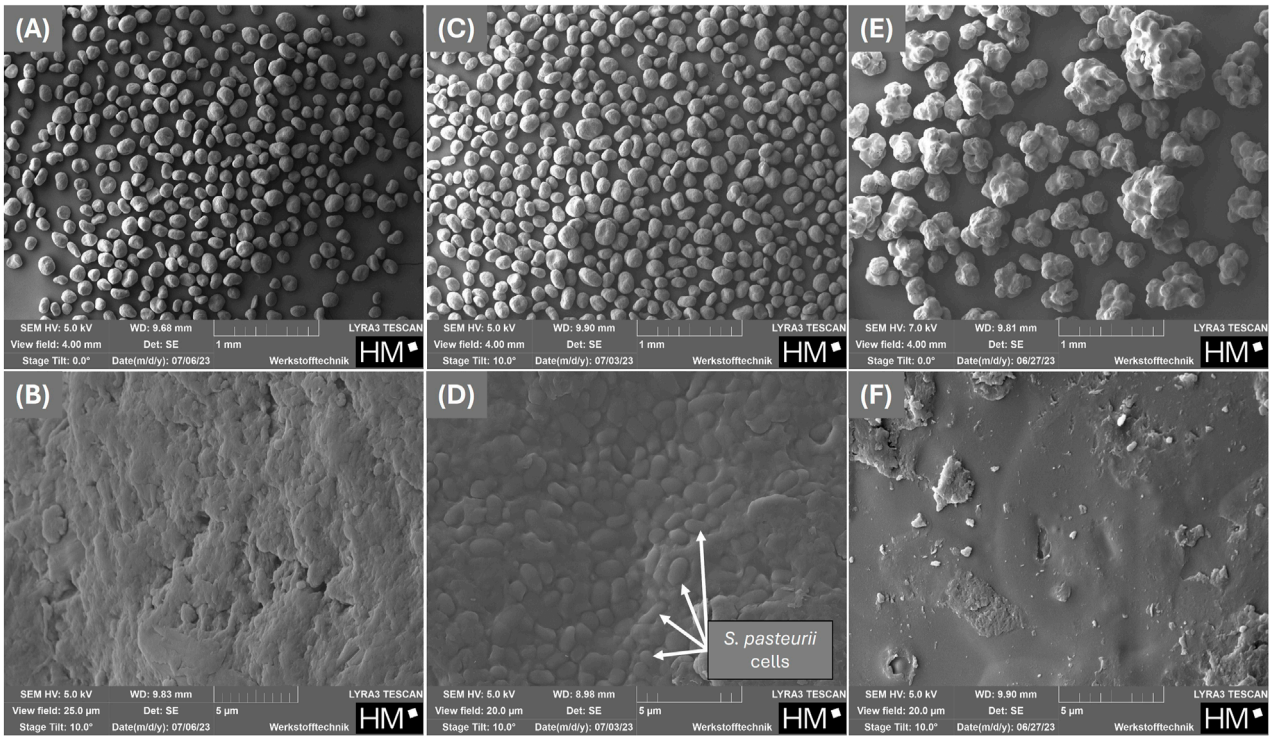


FIGURE 2
SEM-micrographs of the carrier used for fluidized bed drying: microcrystalline cellulose before coating with *Sporosarcina pasteurii* **(A,B)** and after coating with *Sporosarcina pasteurii* without **(C,D)** and with 15% (w/w) maltodextrin **(E,F)** as a protectant.

resulting in 3.10% w/o and 3.08% with maltodextrin. The residual moisture content of the MCC before drying was 4.37%. SEM images of the MCC before the fluidized bed drying process (Figures 2A,B) and after the process without (Figures 2C,D) and with maltodextrin as protectant (Figures 2E,F) were taken. Figures 2A,C,D show images at a magnification corresponding to a field of view of 4 mm, while Figures 2B,D,F show images at higher magnification, with a field of view between 25 $\mu$m and 20 $\mu$m. In combination with the particle distribution of the two powders (Figure 4A), it was clearly visible that the protectant led to the agglomeration of MCC particles, resulting in bigger particles and a more uneven particle distribution. For the MCC coated with bacterial cells w/o protectant, no agglomeration was observed in the SEM images (Figure 2C), as confirmed by the particle distribution of the powder, which was identical to that of the MCC before the drying process (Figure 4A). Furthermore, the individual *S. pasteurii* cells are clearly visible as rod-shaped structures in the close-up images of the coated MCC without maltodextrin (Figure 2D). The cells could not be seen with

the protective agent, as the maltodextrin forms a thick layer over them, making the surface of the MCC appear smoother (Figure 2F) than before coating (Figure 2B). Due to the higher viscosity of the suspension with maltodextrin, it could only be pumped into the fluidized bed reactor at a lower flow rate (Table 1), as there was a risk of nozzle clogging. Therefore, the total experimental time with the protective agent was 6 h, compared to 4 h without it. When comparing the cell viability, analysed by CFU counting, the protectant maltodextrin seems to have had a positive influence on it (Figure 5A). From the MCC coated with bacteria in 9 g/L NaCl and 15% (w/w) maltodextrin, 7,400 ± 31 CFU/mL of a bacterial suspension with an $OD_{600}$ of one were counted. In comparison, on the MCC covered only with *S. pasteurii* without a protectant, just 460 ± 137 CFU/mL were counted on CaSo-urea agar plates. However, the influence of the protectant seems very low when the data are compared to the control sample, a 24 h old *S. pasteurii* culture with an $OD_{600}$ of 1, in which $(1.39 \pm 0.061) \times 10^8$ CFUs/mL were detected. Therefore, it can be concluded that more than 99% of the bacteria did not survive the drying process or were likely damaged too severely to form CFUs. Nevertheless, the powder shows urease activity, see Figure 6.

### 3.1.2 Freeze-drying

After the bacterial suspensions with and w/o protectant were freeze-dried in 50 mL tubes and the resulting dried bacterial mass was crushed, SEM images were also taken here. Figures 3A,C show images at a magnification corresponding to a field of view of 1 mm, while Figures 3B,D show images at higher magnification, with a field of view of 40 $\mu$m. Again, a clear difference was visible between the two powders. Without maltodextrin, the surface of the powders shows only bacterial cells attached to each other (Figure 3B). With maltodextrin, a multitude of small rounded spheres can be observed, along with a smooth coating in between (Figure 3D). Individual bacterial cells were not visible. The weight of the powders in each tube without maltodextrin was 0.48 ± 0.06 g and with maltodextrin 5.20 ± 0.19 g. The residual moisture content of the two powders was 9.85% without maltodextrin and 9.24% with maltodextrin. Similar to fluidized bed drying, maltodextrin had a positive effect on the potential formation of CFUs in the freeze-dried bacteria compared to without maltodextrin. Without protectant, just $(8.37 \pm 0.61) \times 10^6$ CFUs/mL could be counted, with protectant $(2.84 \pm 0.16) \times 10^7$ CFUs/mL. This corresponds to survival rates of 6.02% and 20.46%, respectively, compared to the control sample, a fresh *S. pasteurii* culture. Both powders showed similar initial urease activity (Figure 7).

## 3.2 Storage

Over a period of 92 days all powders were stored under three different conditions (room temperature, 4 °C and −20 °C), and their urease activity was measured once every 7 days for each condition and powder. The results of the urease activity measurements of the fluidized bed dried and freeze-dried powders are shown in Figures 6, 7, without maltodextrin as protectant (A) and with maltodextrin (B). The values of the powders dried with maltodextrin were normalized in relation to the cell dry weight. Therefore, all values were multiplied by a balancing factor, which was determined based on the percentage of the cell dry weight (calculated based on the formula of Lapierre et al. (2020)) and all other substances used, as shown in Table 2. Using the calculated proportions of the different components, the balancing factor was applied to adjust the urease measurement values for the two powders dried with maltodextrin. These values are shown in Figures 6B, 7B. Due to this procedure, the values of urease activity during the storage could be compared for both powders. In addition, the temperature at which the samples were measured with the urease assay was plotted in the graphs to show its influence.

### 3.2.1 Fluidized bed drying

The dried powder using the fluidized bed drying process with a protectant showed a higher urease activity of 7.50 $\frac{\mu S}{cm*s}$, compared to 4.39 $\frac{\mu S}{cm*s}$ when no maltodextrin was used. Over the storage time of 92 days, the decrease in the urease activity was for both powders the highest when stored at room temperature with 28.06% with and 22.02% loss without maltodextrin, as shown in Table 3. The behavior of urease activity of both powders, without or with protectant, looks identical over the storage period (Figure 6). The recognizable peaks in the measured values can be attributed to an increased temperature when measuring the enzyme activity.

### 3.2.2 Freeze-drying

A greater loss of the urease activity after the storage at room temperature and 4 °C was observed in the powder freeze-dried without protectant, as shown in Table 4. Nevertheless, when stored at −20 °C, its enzymatic stability was similar to that of the powder containing maltodextrin, showing only a 21.59% decrease compared to 22.63%. The value of the urease activity directly after drying was higher with maltodextrin compared to without protectant, at 96.44 $\frac{\mu S}{cm*s}$ and 90.31 $\frac{\mu S}{cm*s}$ respectively. As can be seen in Figure 7A, the urease activity decreased most rapidly and strongly during storage for the powder without maltodextrin and storage at 4 °C and room temperature, resulting in a loss of 64.23% and even 67.86%.

## 3.3 Usage for MICP

In order to determine the potential of the various powders for the process of biocementation after storage, sand columns were produced as test specimens. A total urease activity of 40 $\frac{\mu S}{cm*s}$ was set for each sand column, based on the last measured value of the storage period. The results are shown in Figure 8 for the fluidized bed dried (A) and freeze-dried powders (B). The negative controls, consisting of 80 g of pure quartz sand or a mixture of 10 g of MCC with 70 g of quartz sand treated with cementation solution but without bacteria, showed barley measurable UCS measurable UCS (data not shown).

### 3.3.1 Fluidized bed drying

Both powders, with and without protectant, showed urease activity after storage and could still be used for biocementation, as shown in Figure 8. A solidification of the sand was observed in all column areas, comparable to that achieved with a liquid culture without MCC in the sand. The highest strength achieved was 4.35 $\frac{N}{mm^2}$. The decrease in strength from the lower section

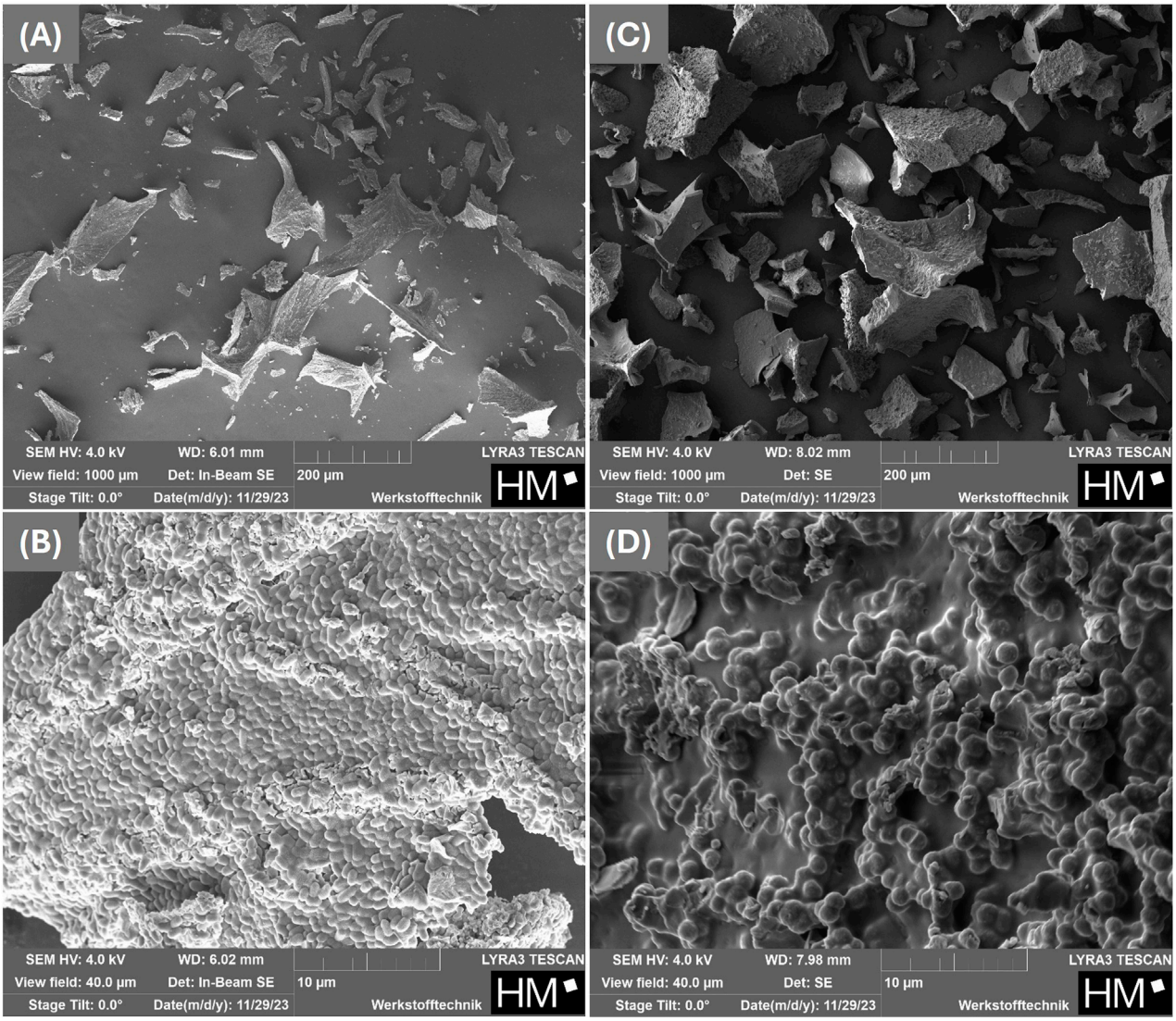


FIGURE 3
SEM-micrographs of the freeze-dried organism *Sporosarcina pasteurii* without **(A,B)** and with 15% (w/w) maltodextrin as protectant **(C,D)**.

(inlet) to the upper section (outlet) of the sand column was identical to that of the control sample. It was noticeable that the control sample, prepared with uncoated MCC and quartz sand, showed a much lower maximum strength of 0.26 N/mm$^2$. For further investigation, SEM images of the tested soil columns were taken from the bottom section. Figure 9A shows an overview of the overall structure. Each sand particle was covered with $CaCO_3$ precipitations on the surface and $CaCO_3$ bridges between particles. The $CaCO_3$ shells, which previously contained sand particles, but which broke out during the measurement or sample preparation, could also be clearly identified. With a larger magnification, a more detailed insight into the resulting microstructures can be achieved, see Figure 9B. All three polymorphs of $CaCO_3$ were visible and identified by their crystal habits: spherulites of needle-like aragonite crystals, rhombohedral calcite, and cauliflower-like vaterite.

### 3.3.2 Freeze-drying

The maximum UCS of 10.81 $\frac{N}{mm^2}$ was reached by the dried bacteria which were covered with protectant and stored at −20 °C. The UCS values, which could be reached with the two freeze-dried powders were nearly three times higher in the first section of the sand columns in comparison to the fluidized bed dried powders. Therefore, the strengths were also higher than the control sample with a liquid *S. pasteurii* culture. However, the decrease in strength from the bottom area (inlet) of the sand column to the top area (outlet) looks similar. The SEM image of the overall microstructure, shown in Figure 10A, revealed a higher density and quantity of precipitates in comparison to soil columns prepared with the powder from the fluidized bed drying process. The individual sand particles were covered with more precipitates of the three $CaCO_3$ polymorphs leading to a stronger interlocking of the microstructure (Figure 10B).

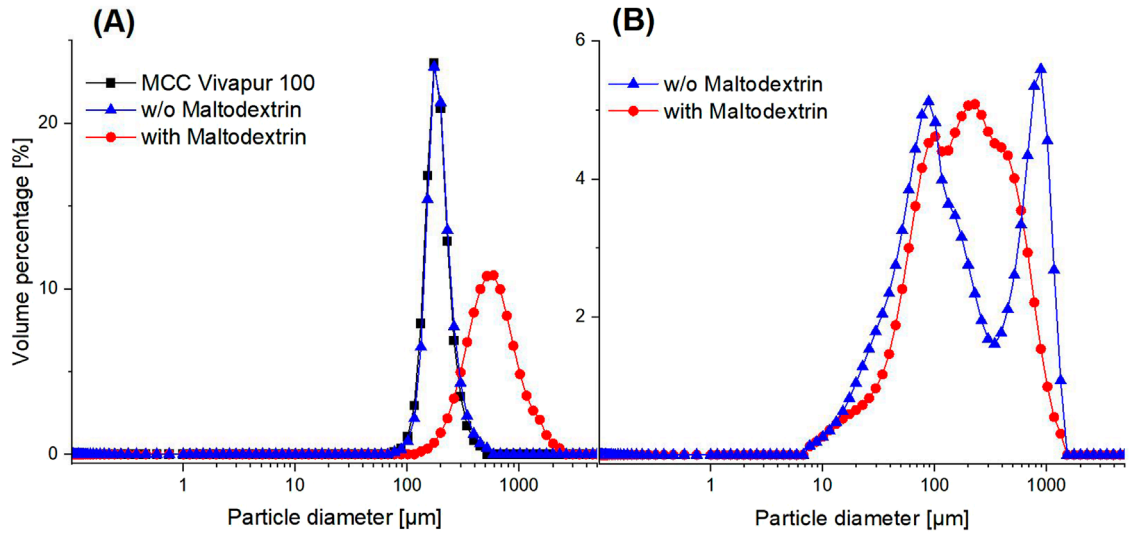


FIGURE 4
Particle size distribution of dried *Sporosarcina pasteurii* using fluidized bed drying **(A)** by coating on microcrystalline cellulose (MCC Vivapur 100) and freeze-drying **(B)**. Both methods were performed without and with the cryoprotectant maltodextrin.

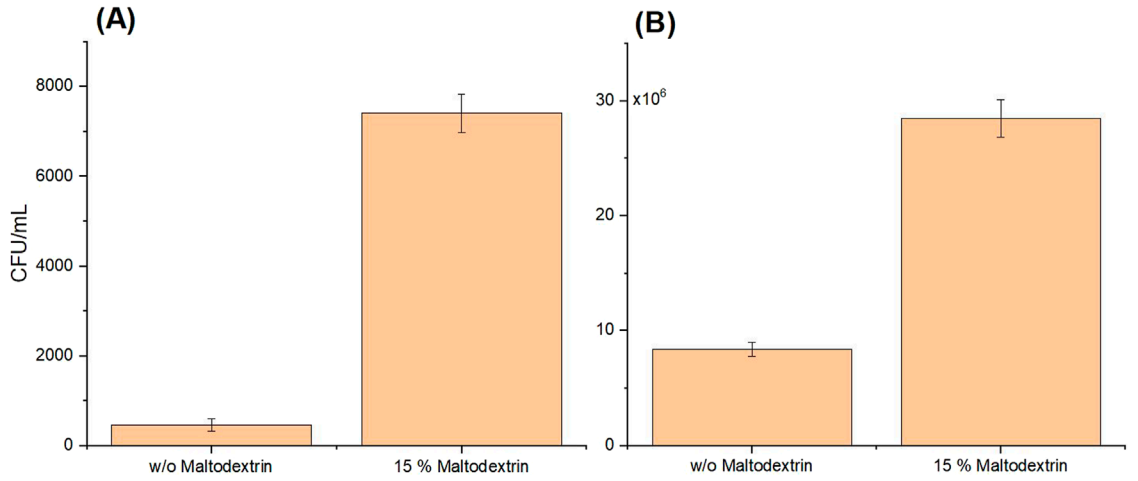


FIGURE 5
Cell viability of *Sporosarcina pasteurii*, tested on agar plates by colony-forming unit (CFU) counts per mL of resuspended powder after fluidized bed drying **(A)** and freeze-drying **(B)**, without and with maltodextrin as a protectant. The plated suspension was prepared from resuspended powder and adjusted to an $OD_{600}$ of 1.

# 4 Discussion

## 4.1 Post-drying sample analysis (residual moisture, image analysis, cell viability)

The low viability of the cells after fluidized bed drying cannot be attributed to the type of spray flow, which consisted of nitrogen. Ghandi et al. (2012) showed that during spray drying, an atomization stage with nitrogen ensures higher survival rates in *Lactococcus lactis* than ambient air alone, due to the prevention of oxidation of the cell membrane by the inertness of nitrogen. It was therefore assumed that this positive effect of nitrogen could also have come into play with fluidized bed drying in the present study. However, after fluidized bed drying, hardly any cells (<1%) were still able to form CFUs (Figure 5A). Nevertheless, there was measurable urease activity, indicating that the urease enzyme was still active after drying. Wang et al. (2024) showed that immobilizing urease on MCC is possible, which could also be seen in the present study with urease containing cells. One theory for the low viability of the cells after fluidized bed drying could be that the residual moisture content of the powder was too low, as it was significantly reduced to 3.08% and 3.10% by post drying, shown in Table 2. Here, the presence of

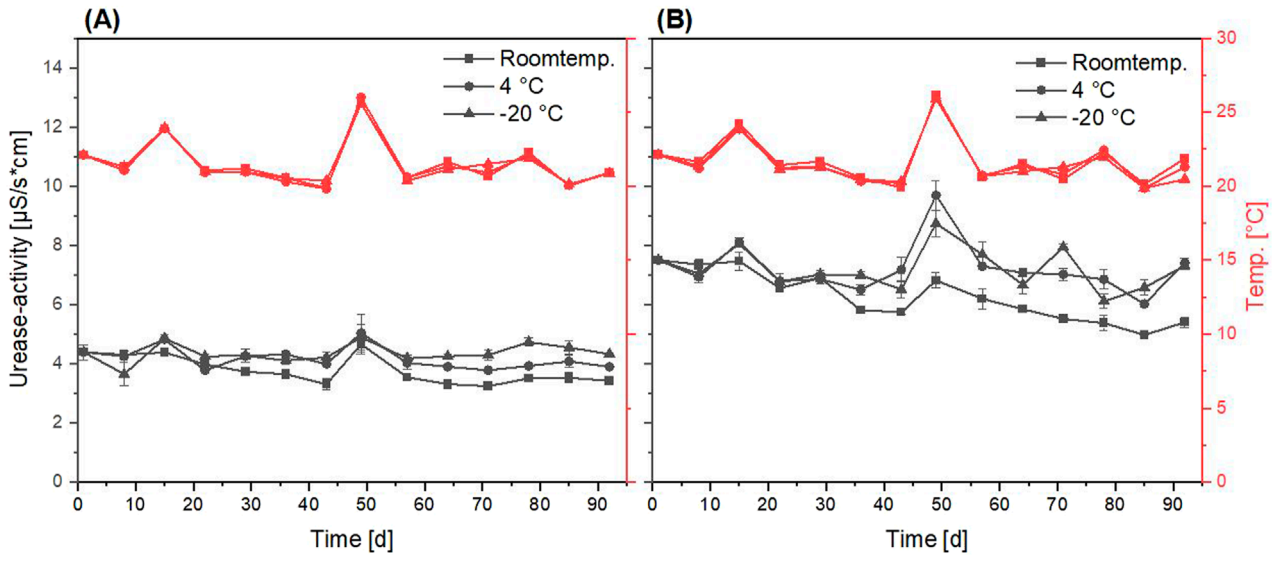


FIGURE 6
Progression of urease activity (black graphs) of fluidized bed dried *Sporosarcina pasteurii* powder over 92 days of storage under different conditions (room temperature, 4 °C, −20 °C) without **(A)** and with **(B)** maltodextrin as a protectant; Temperatures (red graphs) during the conductivity-based measurement of the powders urease activity.

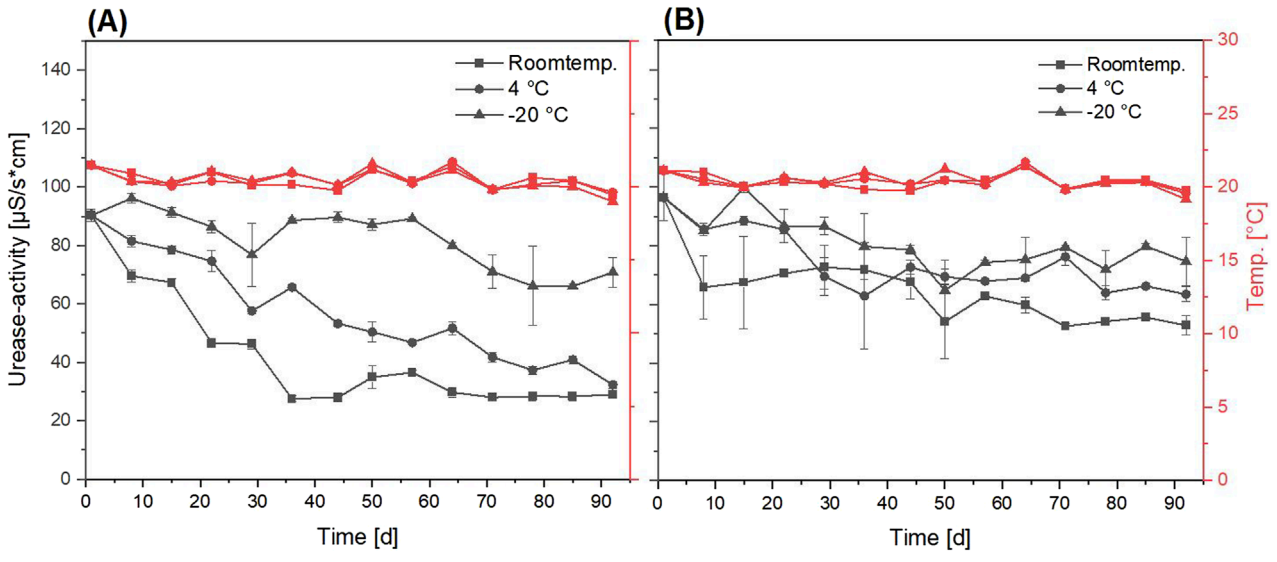


FIGURE 7
Progression of urease activity (black graphs) of freeze-dried *Sporosarcina pasteurii* powder over 92 days of storage under different conditions (room temperature, 4 °C, −20 °C) without **(A)** and with **(B)** maltodextrin as a protectant. Temperatures (red graphs) during the conductivity-based measurement of the powders urease activity.

the cryoprotectant maltodextrin had no influence on the residual moisture. The nozzle used for spraying the bacteria into the drying chamber had a diameter of 0.8 mm, which was comparable to those used in other studies that dried bacteria using fluidized bed drying Bensch et al. (2014), which achieved a significantly higher survival rate of the cells. These findings indicate that the nozzle, as well as the temperatures used and the mass flow rate, which were lower in the present study compared to similar studies Bensch et al. (2014); Stummer et al. (2012); Strasser et al. (2009), can be ruled out as the cause of the low viability.

With freeze-drying, the residual moisture was significantly higher with 9.24% and 9.85% and the viability of the cells was almost 21% (Figure 5B). It has already been reported that a water content that is too high or too low could affect the viability of dried cells. Zayed and Roos (2004) discovered an optimal residual moisture for freeze-dried *Lactobacillus salivarius* cells

TABLE 3 Comparison of initial and final urease activity in fluidized bed dried powder, including percentage loss after storage for 92 days under three different conditions (room temperature (RT), 4 °C, and −20 °C).

| | w/o maltodextrin | | | w/ maltodextrin | | |
|---|---|---|---|---|---|---|
| Urease activity | RT | 4 °C | −20 °C | RT | 4 °C | −20 °C |
| Before storage [$\mu$S/cm∗s] | 4.39 | | | 7.50 | | |
| After storage [$\mu$S/cm∗s] | 3.42 | 3.89 | 4.33 | 5.40 | 7.40 | 7.31 |
| Decrease during storage [%] | 22.02 | 11.31 | 1.44 | 28.06 | 1.38 | 2.58 |

TABLE 4 Comparison of initial and final urease activity in freeze-dried powder, including percentage loss after storage for 92 days under three different conditions (room temperature (RT), 4 °C, and −20 °C).

| | w/o maltodextrin | | | w/ maltodextrin | | |
|---|---|---|---|---|---|---|
| Urease activity | RT | 4 °C | −20 °C | RT | 4 °C | −20 °C |
| Before storage [$\mu$S/cm∗s] | 90.31 | | | 96.44 | | |
| After storage [$\mu$S/cm∗s] | 29.03 | 32.30 | 70.81 | 52.89 | 63.44 | 74.61 |
| Decrease during storage [%] | 67.86 | 64.23 | 21.59 | 45.15 | 34.21 | 22.63 |

between 2.8%–5.6% and Oluwatosin et al. (2022) reached also a lower residual moisture with 2.92% after freeze-drying *Lactobacillus plantarum* cells.

Compared to studies that had also worked with maltodextrin as a cryoprotectant, this viability (also called survivability) was rather low. For *Lactobacillus acidophilus* FTDC 3081 other cryoprotectants, such as skim milk or sucrose, worked better in a freeze-drying process in comparison to 10% or 20% (w/v) maltodextrin, but still better than without Tang et al. (2020). In the present study, too, the cryoprotectant always had a positive influence. Therefore, the low vitality compared to the state of research for other organisms can rather be attributed to the drying behavior of *S. pasteurii*. Ge et al. (2024) tested four different possible cryoprotectants in five different concentrations, each with the most varied vitality results after freeze-drying for the organism *L. lactis* ZFM559. This highlights how diverse and complex it can be to find the right cryoprotectant in the appropriate concentration for a given organism, likely for *S. pasteurii* as well. In the case of *L. plantarum* cells, a decline in cell viability was observed after freeze-drying with water or 10% (m/v) maltodextrin and after storage at room temperature or 4 °C for 12 weeks. However, the decrease was slower at 4 °C and with maltodextrin. Despite this, viability after drying was significantly lower compared to other protectants such as skimmed milk and inulin. Oluwatosin et al. (2022) The drying method of fluidized bed drying resulted in a relative viability of approximately 15% for *L. plantarum* when dried with maltodextrin, whereas freeze-drying led to a higher viability of about 25%. In most studies, viability declined over the storage period, regardless of storage conditions, the presence of protectants, or the drying method used. Protectants only slowed down the rate of viability loss rather than preventing it entirely. Strasser et al. (2009) It can therefore also be assumed that the cell viability of the different powders in the present study will have decreased even further after the storage period of 92 days at room temperature and 4 °C.

When looking at the SEM images (Figures 2E,F; Figures 3C,D), it was noticeable that the powder with maltodextrin had a smooth, non-cracked surface without the bacteria being visible. Majidzadeh Heravi et al. (2022) also described a uniformly smooth surface of the maltodextrin-coated or dried powders. It can therefore be assumed that maltodextrin completely surrounds and embeds the cells. This protected them from further external influences until they were rehydrated again. However, this protection did not appear to be sufficient to protect the cells from a loss of viability if the residual moisture content of the powder was too low.

## 4.2 Storage

When looking at the urease activity at the beginning of storage (Figures 6, 7), the high difference in urease activity per gram of powder can be attributed to the proportion of MCC in the weight of the fluidized bed dried powder. Due to the unknown loss of MCC and liquid not sprayed onto it during the drying process, an exact calculation was not possible. For the freeze-dried powder, this effect was not relevant, due to the directly dried bacteria without any carrier. In comparison to stored liquid cultures of *S. pasteurii* Erdmann et al. (2022); Mehring et al. (2021), the urease activity showed a better stability for every powder, except the freeze-dried powder without maltodextrin (Figure 7A).

The storage conditions of *S. pasteurii* in liquid culture showed varying effects on viability, optical density, and urease activity. After 18 days, no significant impact was observed at −18 °C or 4 °C, whereas at room temperature, viability decreased by more than 80%, optical density was reduced probably due to cell lysis by more than half, and urease activity declined noticeably Erdmann et al. (2022). Similarly, *S. pasteurii* stored at 4 °C in the dark for over 35 days exhibited a 69% reduction in cell viability and a 31% decrease in urease activity after 36 days Mehring et al. (2021). In contrast to previous work, the dried bacteria in the present study were stored under similar conditions but for a longer period (92 days). Compared to the reported stored liquid cultures, the powders, especially with maltodextrin as a protectant and under cool storage conditions (4 °C and −20 °C), showed only a slight decrease in urease activity with a maximum of 34.21% (Table 3 and 4). Storage at room temperature always showed the highest decrease in enzyme activity, but even after 92 days, more than 55% of the initial enzyme activity could be achieved in the settings with maltodextrin as protectant. Enzymes, including urease,

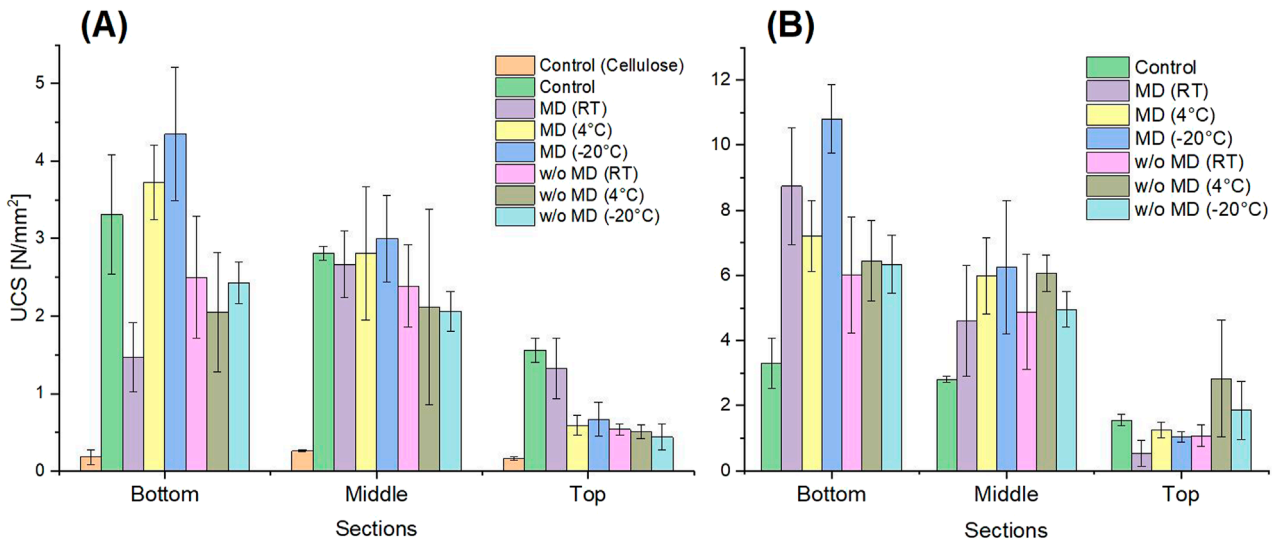


FIGURE 8
Uniaxial compressive strength (UCS) data of the reactivated *Sporosarcina pasteurii* powder dried with fluidized bed drying **(A)** and freeze-drying **(B)**. For all treated sand columns, a total urease activity of 40 $\frac{\mu S}{cm*s}$ was achieved by mixing quartz sand with the dried powder. Control columns were prepared with 25 mL of a 24 h old *Sporosarcina pasteurii* liquid culture, diluted to a total urease activity of 40 $\frac{\mu S}{cm*s}$. One control (orange bar) was prepared by mixing 10 g microcrystalline cellulose (MCC) and 70 g quartz sand, the other control (green bar) consisted out of 80 g quartz sand.

are susceptible to denaturation throughout production, storage, and application. Environmental factors such as temperature, pH, chemical agents, or ionic strength can influence their activity Iyer and Ananthanarayan (2008). In general, enzymes exhibit greater stability at lower temperatures Alev et al. (2019), which is also confirmed by the results of the present study.

In comparison to the study of Tuttle et al. (2025), in which the lyophilized *S. pasteurii* cells were stored in plastic bags at 25 °C for up to 3 months, just the viability was monitored, not the urease activity. CFU tests showed a significant 3-month decline without cryoprotectant, while counts with cryoprotectant were approximately one log higher. The occurrence of cell and enzyme lysis during storage might also have played a role in the present study and could be another reason for the decrease in urease activity of the freeze-dried powder without cryoprotectant.

## 4.3 Usage for MICP

Due to the low viability of the *S. pasteurii* cells after the fluidized bed drying process, it can be assumed that using this powder for biocementation was no longer a MICP process but rather an enzyme induced carbonate precipitation (EICP) or a mixture of both principles. For the freeze-dried powders, the viability was higher with almost 21%, therefore both, MICP and EICP, probably occurred with the freeze-dried powder. In general, the enzyme urease is described as an intracellular enzyme Mobley et al. (1995), and in the organism *S. pasteurii*, urease is also present intracellularly Ma et al. (2020). Due to the low viability, cell damage must be assumed, such as damage to the cell wall, which could lead to the release of intracellular urease. This extracellularly available urease would still be able to catalyze urea hydrolysis, thereby enabling carbonate precipitation even in the absence of fully viable cells.

A key advantage of the approach used in the present study were the exceptionally high UCS values achieved. Reviews featuring numerous UCS values indicate that the most reported values (Zhang et al., 2025; Fu et al., 2023; Tong et al., 2021), although derived from liquid cultures and therefore not directly comparable, are generally lower than those obtained in the present study. This indicates that the treatment method achieves remarkably high compressive strengths in sand columns even without optimization. Compared to a liquid culture with the same total urease activity, higher strengths were achieved with the different dried powders. This could be attributed to the fact that the enzyme and the microorganism were less likely to be washed out by the cementation solution, consisting of calcium ions and urea, when present in powdered form or coated on MCC. Adsorption studies have already demonstrated that microbial washout consistently occurs when MICP was conducted using liquid cultures of *S. pasteurii* Sang et al. (2023); Tobler et al. (2014); Hanisch et al. (2024). As a result, the urease activity in the sand column decreases with each flow of the cementing solution, leading to lower strengths of the control (green bars), as shown in Figure 8. Similar observations regarding the inhomogeneous distribution and strength reduction of the sand columns with distance from the solution inlet were reported by Hanisch et al. (2024). It can be attributed to the consumption of calcium ions in the initial areas of the sand column due to the MICP process, followed by the formation of $CaCO_3$. Higher calcium concentrations in the cementation solutions might potentially reduce this effect.

Furthermore, it is important to emphasize that, in theory, all sand columns in the present study, prepared with the dried powders, should exhibit the same strength, as they were subjected to

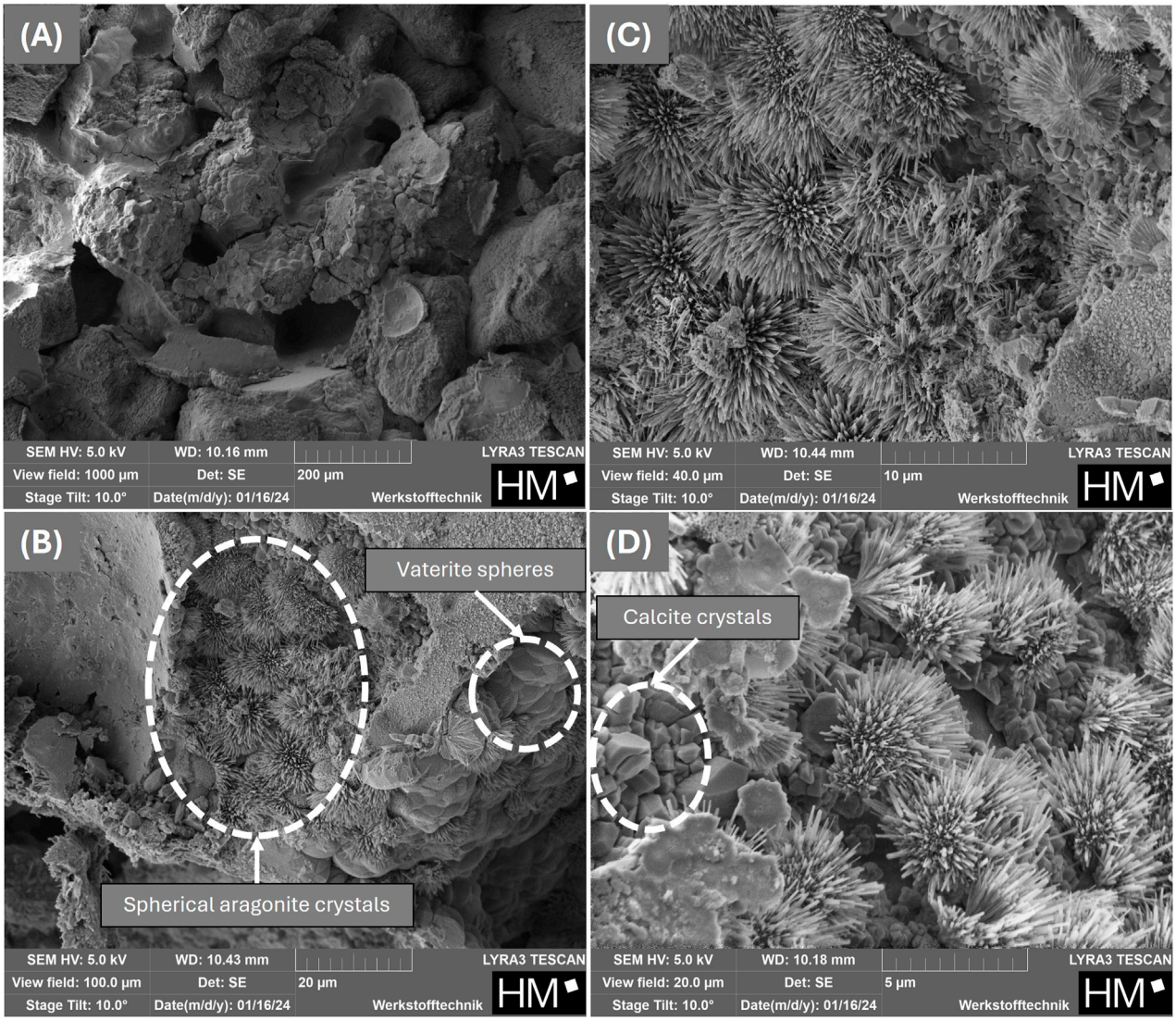


FIGURE 9
SEM-micrographs of fragments from the bottom section of cured sand columns with increasing magnifications from **(A-D)** produced with the powder obtained from fluidized bed drying with maltodextrin.

identical urease activity. Within the respective drying method, this also applies approximately, no matter with or without protectant. Therefore, an influence of the protectant on the strength of the sand column can be excluded. However, there was a big difference in the strengths when comparing the two powders produced in different ways. With UCS values in a range of 1.47–4.35 $\frac{N}{mm^2}$ for the fluidized bed powder, they were nearly two times lower in comparison to the freeze-dried powder with a range of 6.01–10.81 $\frac{N}{mm^2}$. This suggests an influence of the MCC, which also applies to the control sample made of quartz sand and MCC not coated with bacteria. This only achieved a strength of 0.18 $\frac{N}{mm^2}$, without MCC the control reached a significantly higher strength of 3.31 $\frac{N}{mm^2}$. The negative influence of MCC cannot be attributed to the inhibition of urease activity. Conductivity measurements assessing the enzymatic activity of *S. pasteurii* in the presence of varying amounts of MCC showed no impact on urease activity (data not shown). Furthermore, urease itself Lv et al. (2018); Wang et al. (2024) or spores of urease-producing organisms Xu et al. (2024) have been immobilized on various types of cellulose in multiple studies, without any reported inhibition of the enzyme. The zeta potential of uncoated MCC (−12.5 mV Wang et al. (2024)) is also unlikely to play a role, as both quartz sand and *S. pasteurii* carry even more negative charges, making repulsion effects more likely in those cases. A possible explanation for the observed decrease in strength is the high amount of added MCC, approximately 10 g within a total sand column weight of 80 g. MCC particles have a very round shape, which lacks edges and has a smoother surface compared to sand grains, potentially reducing the overall stability of the structure. This is partially supported by a study of Song et al. (2022) et al., which observed higher UCS values for angular compared to spherical particles within the 0.85–0.425 mm size range, using a largely similar experimental setup to the present study. However, when the particle

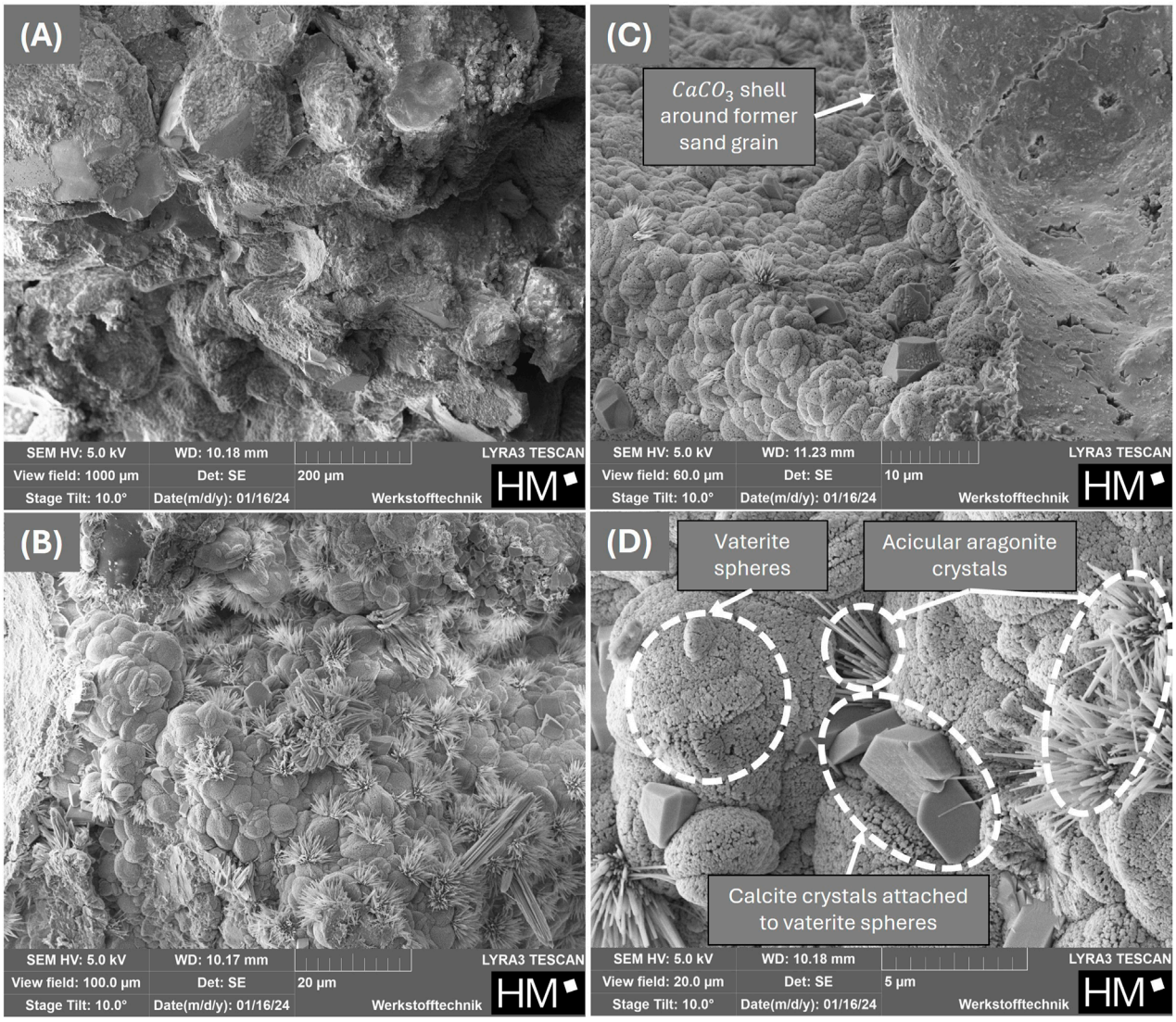


FIGURE 10
SEM-micrographs of fragments from the bottom section of cured sand columns with increasing magnifications from **(A-D)** produced with the powder obtained from freeze-drying with maltodextrin.

size distribution decreases to 0.250–0.180 mm, the UCS values were significantly higher for spherical-shaped particles than for angular ones. Additionally, the surface roughness affects $CaCO_3$ precipitation on different kind of particles and the UCS values as well, as stated by García-González et al. (2017); Erdmann et al. (2024). This effect may have influenced the UCS values in the present study, as the roughness of MCC and quartz sand particles were different and so could have impacted both, $CaCO_3$ formation and particle bonding.

In the study by Tuttle et al. (2025), sand columns were treated up to twelve times with a cementation solution (0.33 M urea, 0.33 M $CaCl_2 \cdot 2H_2O$), following an initial treatment with lyophilized *S. pasteurii* cells resuspended in deionized water. The maximum UCS values reached were approximately 2.1 $\frac{N}{mm^2}$, which is much lower than the UCS values achieved in the bottom and middle sections of the sand columns in the present study using the freeze-dried powder. Terzis and Laloui (2018) and Terzis and Laloui (2019) were able to reach UCS values with resuspended lyophilized *S. pasteurii* cells up to 11.3 $\frac{N}{mm^2}$. Valencia-Galindo et al. (2021) reported that treatment with freshly cultivated bacteria on sandy silt generally resulted in better performance compared to using a resuspended powder of lyophilized MICP-capable bacteria. These different results suggest that not only the form of the cells (powdered or dissolved) but also the experimental conditions such as the number of treatments, the substrate properties, and the solution composition can strongly influence the resulting soil strength. Therefore, a fundamental comparison of the experiments from the literature described above is difficult, as all these studies and the present study use different methods and soils to be treated.

SEM images (Figures 9, 10) of the different $CaCO_3$ precipitates closely resemble those reported in previous studies Rodriguez-Blanco et al. (2011); Chen et al. (2024); Xu et al. (2020); Ševčík et al.

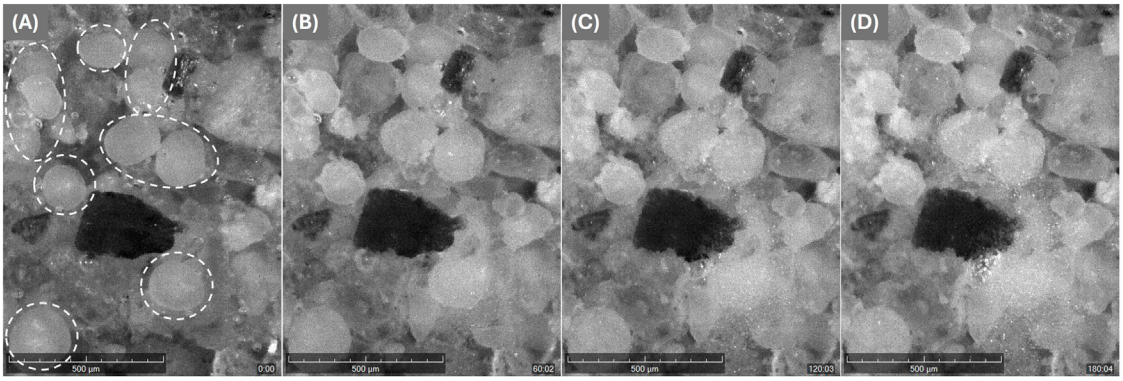


FIGURE 11
Images from the Crystallization System (Technobis), show the resulting $CaCO_3$ precipitates formed by fluidized bed dried *Sporosarcina pasteurii* coated on microcrystalline cellulose without maltodextrin, mixed with quartz sand, and covered with cementation solution. Image **(A)** was taken at time 0 (prior to adding urea and calcium), with images **(B-D)** following at 1-h intervals. The microcrystalline cellulose (MCC) particles are marked in Figure **(A)**.

(2018). Based on these, the precipitates do not appear to be amorphous $CaCO_3$ but rather consist of all three possible crystalline forms. This might be due to the storage time of the treated sand columns for 2 weeks at 40 °C. Chen et al. (2022) reported that the transformation of amorphous $CaCO_3$ into calcite crystals begins after just 24 h and is nearly complete after 54 h. The kinetics of mineralization reactions catalyzed by the enzyme urease depend on various parameters, such as $CaCl_2$ or ammonium ion concentration Hu et al. (2021), but were not analysed further in the present study.

Figure 11 illustrates the formation of $CaCO_3$ precipitates over time from the particles coated with *S. pasteurii* and the bonding of individual particles. The MCC acts as nucleation sites from which the precipitates, white shading around the MCC particles, spread and form bonds to the surrounding particles. A similar process occured in the sand columns, which were saturated with fresh cementing solution for 100 min before a new solution was introduced. This confirms that the precipitates originate almost exclusively from the MCC and that the urease enzyme was not directly detached from it, as the pump operated at a very low flow rate of 0.25 mL/min.

## 5 Conclusion

The study investigates the possibility of drying the microorganism *S. pasteurii*, which is frequently used in the field of MICP, using two different techniques: the commonly used freeze-drying and fluidized bed drying, both with and without the protectant maltodextrin. As a first study, these two methods were examined with regard to long-term storage of cells for 92 days under different conditions and their subsequent application in soil stabilization, with the following main results.

- Maltodextrin showed a positive effect on cell viability and urease stability for both drying methods. For fluidized bed drying it tended to make the entire spraying process longer due to higher viscosity and led to a powder with strong agglomeration. In the case of freeze-drying, the protectant had a much more positive effect.
- The freeze-dried powder with maltodextrin showed the highest cell viability (ability to form CFUs) with almost 21% compared to the control (fresh liquid culture). After fluidized bed drying, the cell viability was less than 1%. Nevertheless, measurable enzyme activity was detected immediately afterwards and also after 3 months of storage for all powders.
- The storage temperature of the different powders (room temperature, 4 °C, −20 °C) affected the urease activity over time in both cases. At −20 °C, the largest decrease in urease activity was no more than 22.63% for the freeze-dried *S. pasteurii* cells with maltodextrin. During storage at room temperature or 4 °C, the decrease in urease activity was greater, with maximum losses of 67.86% and 64.23% for the freeze-dried powder without the addition of maltodextrin.
- Each powder, regardless of the drying or storage method, increased the strength of sand columns through the MICP process by up to 10.81 $\frac{N}{mm^2}$, compared to an initial strength of 3.31 $\frac{N}{mm^2}$ within the same column range. Even without further optimization of the drying and MICP process, above average compressive strengths for sand columns could be achieved compared to the literature with liquid cultures. MCC itself seemed to have negative effects on the UCS, as the controls with and without MCC and liquid cultures showed low strength values, with a maximum of 0.26 $\frac{N}{mm^2}$ and 3.31 $\frac{N}{mm^2}$, respectively. The presence of maltodextrin had no effect on soil compaction in relation to UCS.

Fluidized bed drying can have significantly lower costs, with only 8.8% fixed costs and 17.9% production costs compared to freeze-drying (Santivarangkna et al., 2007). In addition to the high variability of the carrier materials (size, materials, particle distribution), it is a highly efficient alternative to freeze-drying. Moreover, the present study clearly demonstrates, even without process optimization, that dried *S. pasteurii* bacteria can be stored for extended periods and successfully reused for soil stabilization.

In addition, dried bacterial powders offer significant advantages not only for storage but also for transport to specific application sites. The reduced weight and volume of dried bacteria in comparison to liquid cultures lead to lower transportation costs, making large-scale MICP applications more feasible and economically viable. Furthermore, drying allows for applying the cells in higher concentrations, potentially enhancing their effectiveness in MICP processes and improving overall performance in practical applications.

The present study confirms that fluidized bed and freeze-drying enables storage of *S. pasteurii* even at room temperature without the need for a protective agent, highlighting its suitability for practical MICP applications and supporting large-scale implementation—thus making MICP more easy and broadly applicable in the construction and geoengineering sectors.

Further optimization of the drying processes, including the investigation of various cryoprotectants and their concentrations, could significantly improve storage stability. In addition, alternative drying methods, such as spray drying, may provide promising results for preserving bacteria used in MICP applications. Moreover, reactivation protocols—such as the composition of the recovery media and pre-hydration steps—are also critical factors that can influence the viability and performance of dried cells upon reuse.

## Data availability statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

## Author contributions

PH: Writing – original draft, Investigation, Conceptualization, Methodology. MP: Investigation, Writing – review and editing. CE: Resources, Writing – review and editing. SK: Conceptualization, Writing – review and editing. TM: Resources, Conceptualization, Writing – review and editing. RH: Funding acquisition, Supervision, Conceptualization, Writing – review and editing, Project administration.

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## Conflict of interest

Authors SK and TM were employed by Wacker Chemie AG.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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