

# Analysis of capillary absorption properties of porous limestone material and its relation to the migration depth of bacteria in the absorbed biomineralizing compound

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## Abstract

In the present paper migration characteristics of *Bacillus cereus* in porous limestone, as well as capillary absorption and elevation properties of porous limestone were analyzed. For the measurements two newly designed measuring methods were used: a combined capillary absorption – capillary elevation test, and printing-on-agar technique of treated specimens on *cereus*-selective agar plates. As result of the experiments relation between the capillary absorption and capillary elevation coefficients was validated, and physical meaning of the free water-content was clarified. Moreover, both the printing-on-agar technique and the combined capillary absorption – capillary elevation test turned out to be efficient methods for the analysis of the migration height of the bacteria. Combined results of these tests showed, that elevation height of the bacteria can be enhanced upon application of more liquid curing compound. It was also found, that the amount of the curing compound can be partly substituted with the post-addition of water without significant decrease of the migration height.

## Keywords

biomineralization · porous limestone · *Bacillus cereus* · migration of bacteria · capillary elevation · capillary absorption

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## 1 Introduction

Nowadays there are a handful of research groups engaged in the development of biomineralization for conservation and cementation purposes [1]. Biomineralization is the capability of different bacteria strains to produce calcium-carbonate crystals in adequate environment. The adequate environment can be ensured not only naturally, but also artificially, by mixing bacteria together with organic CaCO<sub>3</sub>-sources in a liquid compound [2], or printing bacteria on adequate solid agar [3]. This method is called microbially induced calcium carbonate precipitation (MICP). Since porous materials are capable to soak liquidized biomineralizing compounds, crystal-formation can be initiated inside the material, too. This way damaged, inner, thus hardly accessible parts of lime-based porous construction elements will be treatable and repairable in a highly compatible way.

Until now, microbially induced calcium carbonate precipitation has been analyzed and evaluated by different research groups from several aspects, both regarding the curing compounds and the porous material. One of the minor fields of research on biomineralization is evaluation of the effective depth of the treatment, i.e. the maximum depth in which production of bacterial calcium carbonate occurs. Determination of the effective depth yet has been based only on the evaluation of empirical results obtained from biomineralizing experiments, and on hypotheses. As an example, type of microorganism was believed to have consequences on the penetration depth of the treatment and on the possibility of biofilm formation. The use of bacteria showing gliding motility could result in a larger penetration depth [4]. However, this hypothesis has not yet been confirmed by experimental results.

Intense microbial carbonate precipitation can only occur in an environment, where bacteria are present. Without bacterial contribution, only chemical precipitation occurs, in a much lower extent [5], [6]. Until now, indicator of the effective depth of the treatment was the presence and distribution of the newly formed crystals. These crystals were first analyzed by scanning electron microscopy (SEM) on porous limestone by Rodriguez-Navarro et al. [7]. They observed calcium carbonate crystals down to nearly 1 mm in the porous system of the stone immersed in M-

3 medium. Their other samples in inoculated M-3P medium developed coarse sparitic cement composed of calcite rombohedra, fully covering the pore walls down to a depth of 0.5 mm. Jimenez-Lopez et al. [8] reported formation of vaterite globule inside a pore of porous calcarenite stone immersed in the same, aforementioned medias at a depth of 3 mm, determined by scanning electron microscopy. De Muynck et al. [4] were able to observe precipitation at up to 1 mm depth by means of thin section analyses (by optical microscopy) of Euville limestone treated by immersion with urea and calcium chloride. However, they reported that such analyses are rather difficult because of the fact that the newly formed layer has the same composition as the stone matrix. In this case, the bacterial origin of the precipitates could be observed because of the presence of some bacterial remains. Séverine et al. [9] tested the Calcite Bioconcept method on gypsum plaster, and estimated the depth evolution of the calcite and salts phases from the variations in integrated intensities of X-ray absorption. They reported that calcite/gypsum and salts/gypsum ratios decrease with depth, demonstrating that salt and calcite are present only in the superficial layer. Validity of their ratios held only for the few first  $10\ \mu\text{m}$  from the surface. Eventually, De Muynck et al. [10] analyzed the effective depth and the bacterial products with help of microtomography (MCT) on five different types of porous limestones. With help of the MCT they reported effective depth between  $300\ \mu\text{m}$  up to 2 mm, dependent on the stone type, growing with the presence of macropores. However, they did not verify, whether the visualized precipitation is calcium carbonate or leftover of organic materials.

Results associated with calcium carbonate precipitation recorded during the above mentioned analyses seem to be rather difficult to verify due to the presence of large amount of organic matter introduced into the stone. For this reason, as well as the fact, that intense microbial calcium carbonate precipitation requires the presence of adequate bacteria, indirect estimation of the effective depth is possible by determination of the distribution of the bacteria inside the porous matrix. Moreover, since bacteria and organic components are carried into the porous matrix of the stone by capillary absorption of the curing compound, extensive preliminary analysis of the absorption characteristics of the stone is desirable for better understanding the achieved distribution of the bacteria.

The aim of the present study was to evaluate the migration properties of bacteria during the absorption process of a curing compound, by relating them to the extensively evaluated capillary absorption properties of the treated material, porous limestone of Sós-kút. Measurements were performed by employing two newly designed techniques:

- 1 printing of treated specimens on agar plates for the indirect estimation of the effective depth, and
- 2 combined capillary absorption and elevation test. By combination of the measured results obtained with the two methods

we supposed to understand and explain the nature of bacterial migration inside the porous limestone.

## 2 Materials

### 2.1 Stone material and preparation of the samples

In the present experiment porous limestone material was used. The origin of the limestone is the Sós-kút quarry in Hungary. Out of the different fabrics of Sós-kút limestone the oolitic type was chosen, which is highly porous, up to 40 v/v % total porosity. Altogether 13 cylindrical specimens were drilled out of masonry blocks, with radius of  $36.84 \pm 0.07\%$  mm and height of  $89.8 \pm 0.78\%$  mm.

### 2.2 Biomineralizing compound and microbes

In the present experiment the biomineralizing compound was received from the French Calcite Bioconcept company. Ingredients of the compound are the bacteria *Bacillus cereus* in lyophilized form, the BioCal (mixture of the nutrients and the organic materials as  $\text{CaCO}_3$  - source) in form of a powder and finally distilled water. The biomineralizing compound was prepared five times in different volumes, as it is explained in 3.2. Dosage of the BioCal was 25 mg/ml, solved in distilled water. Only the first compound was inoculated with 5 mg/ml lyophilized bacteria. Preparation of the first dose of compound and inoculation was performed 20 hours before the treatment, i.e. application into the specimens, in non-sterile conditions. During these 20 hours the bacteria multiplied by consuming the organic nutrients. The liquid compound was stored in a closed plastic box at  $20\ ^\circ\text{C}$ .

### 2.3 Agar plate

For printing the specimens the MERCK Cereus-Selective Mannitol - Egg-yolk - Polymyxine - Agar was used, consolidated with agar-agar. Indicator of the *Bacillus cereus* on this agar is the pink color of the originally red agar under the grown bacteria colonies. For printing the specimens on agar plates the selective agar was poured into closeable plastic containers with open inner surface area of about  $10 \times 15\ \text{cm}$ . This surface area was large enough to print the whole surfaces of the cylinders on it by rolling the specimens over on the top of the agar. About 120 ml of selective agar were poured into each of the 13 containers, and they were stored closed until the printing at  $8\ ^\circ\text{C}$  in a refrigerator.

## 3 Methods

### 3.1 Mass properties of the limestone

Real density and total porosity of the specimens were measured according to the MSZ EN 1936:2007 (E) standard 'Determination of real density and apparent density and of total and open porosity' standards, respectively. Constant water content ( $\omega_c$ ) of the specimens was measured by sinking the specimens in water (water-level was set 15 cm above the top of the specimens) in a bowl for one week, which was long enough for con-

stant saturation (weight-difference is lower than 0.1 m/m % of the mass of the specimen in 24 hour intervals). After saturation, apparent porosity was derived from the constant water-content by dividing the volume of the absorbed water with the volume of the specimen. Volume of the water was calculated from its mass by dividing it with the density of water at 24.5 °C. Water content was measured during the capillary absorption, too. Firstly at  $h_{max}$ , when the front of the elevation reached the top of the specimen, water content can be determined as follows:

$$w_{h,max} = \frac{m_{w,h,max}}{\rho_w} \cdot \frac{1}{V_{spec}} \quad (1)$$

where  $w_{h,max}$  is the water content of the specimen in  $m^3/m^3$ ,  $m_{w,h,max}$  is the mass of the absorbed water at in grams,  $\rho_w$  is the density of water at 24.5 °C in  $g/cm^3$ , and  $V_{spec}$  is the volume of the specimen in  $cm^3$ . Secondly, water content of the specimens was further measured for a few minutes in order to determine whether mass of the absorbed water raises significantly or not after the capillary elevation reached  $h_{max}$ . End of the measurement was determined empirically, and the belonging water content was denoted with  $w_t$ . This way three water contents were recorded, which were expected to be in a relation:  $w_{h,max} < w_t < w_c$ . Measurements were carried out at  $24.5 \pm 0.5$  °C.

### 3.2 Combined capillary absorption and capillary elevation test

Combined capillary absorption and capillary elevation test is a new method designed to measure the mass of the absorbed water and the height of capillary elevation in real time parallel to each other, on the same specimen. In case of materials with high water absorption coefficient (such as porous limestone) this measurement cannot be performed by recording elevation height and measuring the changing mass separately on a balance, since water is likely to rise a few millimeters while measured on the balance. For the parallel measurement the following setup was designed: specimens were hanged in vertical position on a hook of an Archimedes-balance, respectively. Then two plastic rulers with millimeter scale were mounted on the two sides of the specimens, along their length (see Fig 1.). This was necessary since the water-front of the absorption seldom raises parallel to the plane of the water surface. However, in case of a cylinder, average elevation height is the mean value of the two elevation heights measured on the two sides of the specimen. Absorption was initiated by pouring water into a bowl under the hanged specimen until it reached the bottom of the cylinder. Continuous water supply was either ensured by the layer of water attached to the bottom of the specimen due to the surface tension of water linking the surface of water to the specimen. Moreover, sinking of the specimen hanged on the balance followed the decrease of the water in the bowl. Sides of the cylinder were not protected against evaporation, since the gap between the wrapping material and the surface of the specimen could have acted as a

macro pore, influencing the measurement. Masses in time were recorded by projecting the digital screen of the balance into the sight of camera with help of mirrors, as it can be seen in the bottom of Fig. 1.

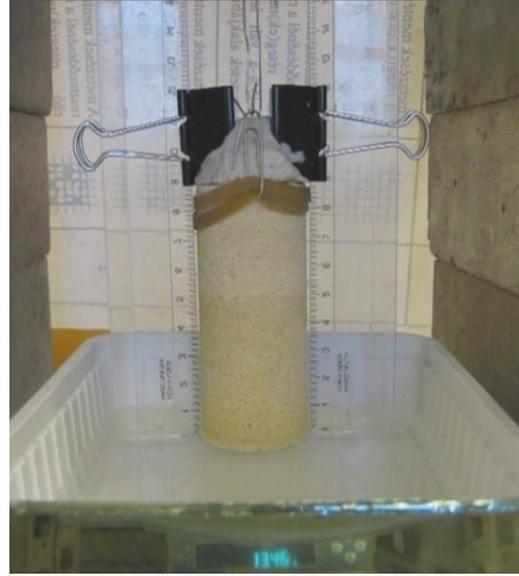


Fig. 1. Testing setup for the capillary elevation measurement

Measured mass of the absorbed water was corrected both with the effect of the evaporation and the mass of the water attached continuously to the bottom of the specimen during the elevation due to the surface tension. Effect of the evaporation was approximated as follows. Rate of evaporation,  $r_e$  is a function of the changing of the evaporating surface area (in case of a cylinder:  $2r \cdot \pi \cdot h$ ), which is linearly proportional to the capillary elevation height. Its maximum value,  $r_{e,max}$  was measured at the end of the elevation process, when the capillary elevation reached the top of the specimen (at  $h_{max}$ , here  $\sim 90$  mm). Maximum value of the rate of evaporation,  $r_{e,max}$  was determined as  $\frac{m}{t \cdot h_{max}}$ , where  $m$  is mass of the evaporated water in grams,  $t$  is the time in minutes, and  $h_{max}$  is the height of the specimen in mm. When the water-front reached  $h_{max}$ , the specimen was placed on a balance, and weight loss due to the evaporation was measured in time. Then the total weight loss in time was calculated as follows:

$$m_{evap}(t) = r_{e,max} \cdot \int_{T_1}^{T_2} h(t) dT \quad (2)$$

where  $m_{evap}(t)$  is the mass of water evaporated during the absorption process (between  $T_1$  and  $T_2$  in min) in grams, and  $h(t)$  is the function of capillary elevation in of time, determined from the measured data.

The whole sorption process was recorded with a digital camera (in a 640 x 480 pixels resolution) until the water-front reached the top of the specimen. Instantaneous elevation heights were determined with help of the videos, and instantaneous speeds of absorption were calculated and evaluated in function of the elevation height. During the absorption test the recorded values were the elevation height ( $h$  in mm) of the water-front in the limestone, the mass of the absorbed water ( $m$  in g), and

the time ( $t$  in s). The measurements were taken about 15 - 20 times in different heights along the length of each limestone sample. Elevation heights in time were read by naked eye from the videos with an accuracy of 0.5 mm.

First of all, the capillary absorption coefficients ( $k$ ) of the specimens were determined in  $\frac{kg}{m^2 \cdot \sqrt{s}}$  by processing the videos. For this purpose calculation method of the standard MSZ EN 1925:2000 was used for the measured values as follows:

$$k = m/A \sqrt{t}$$

where  $m$  is the volume of the absorbed water in kilograms,  $A$  is the absorbing surface in  $m^2$ , and  $t$  is the time in seconds.

After this, height of the elevation ( $h(t)$ ) was evaluated in time. According to [11], capillary elevation in a capillary tube with discrete pore-radius follows a similar trend in time, than capillary absorption, since it is also proportional to  $\frac{1}{\sqrt{t}}$ , as follows:

$$B = \frac{h(t)}{\sqrt{t}} \quad (3)$$

where  $B$  is the capillary elevation coefficient of a pore with a given radius in  $\frac{m}{\sqrt{h}}$ ,  $h(t)$  is the instantaneous height of capillary elevation in m, and  $t$  is the time in hours. According to our measurements on the velocity of capillary elevation performed on porous limestone specimens with a complex pore-system [12], the velocity was found to decrease in time as follows:

$$v(t) = c \cdot \frac{1}{h(t)} + d \quad (4)$$

where  $v(t)$  is the velocity of capillary elevation in time in (mm/s),  $h(t)$  is the instantaneous height of the elevation, and  $c$  and  $d$  are coefficients calculated to range from 14.5 to 30.43  $mm^2/s$  ( $c$ ) and from 0.08 to 0.06 mm/s ( $d$ ). Since  $v(t) = \frac{h(t)}{t}$ , hence, according to Eq. 4

$$c \cdot \frac{1}{h(t)} + d = \frac{h(t)}{t},$$

and by neglecting the value of  $d$  around zero

$$\sqrt{c} = \frac{h(t)}{\sqrt{t}} \quad (5)$$

which suggests, that  $h(t)$  is proportional to  $\frac{1}{\sqrt{t}}$  in complex pore systems, too. Therefore capillary elevation coefficient of the complex pore systems of the porous limestone was denoted with  $e$  (in  $\frac{m}{\sqrt{s}}$ ), and evaluated as

$$e = \frac{h(t)}{t} \quad (6)$$

Eventually, relationship between the mass of the absorbed water and the height of the capillary elevation were evaluated. According to [11], capillary elevation coefficient ( $B$ ) is proportional to the capillary absorption coefficient ( $A$ ) as follows:

$$B = \frac{A}{\rho_w \cdot u_f} \quad (7)$$

where  $\rho_w$  is the density of the absorbed water in  $kg/m^3$ , and  $u_f$  is the maximal free water-content in  $m^3/m^3$ . Using our notation

equivalent of  $A$  is  $k$  (calculated in unit  $\frac{kg}{m^2 \cdot \sqrt{s}}$ ) of  $B$  is  $e$ , and of  $u_f$  is  $w$ . However, maximal free water-content is not defined exactly. Lieboldt refers to it as “maximal absorbable quantity of water”, which suggests, that it is equivalent to constant water content [13]. In the view of this our aim was to find the equivalent of the maximal free water-content out of the three water contents defined under 3.1. by calculating the  $u_f = \frac{e}{k \cdot \rho_w}$  ratios in  $m^3/m^3$ , and comparing them to the measured water-contents, respectively.

Eventually, velocity of the water-front ( $v$  in mm/s) belonging to the migration heights of bacteria in the specimens was calculated by dividing the instantaneous elevation height with the elapsed time.

### 3.3 Biodeposition treatment procedure

The performance of the treatment procedure was based on the procedure of the Calcite Bioconcept Company, and took six days. The bacteria inoculated compound was prepared on Day 1 (0 hour). 14 hours later, on Day 2, the compound was applied into the stone specimens by means of capillary absorption from Petri-dishes. Application of the non-inoculated treating compounds onto the treated area, i.e. further feeding the bacteria was performed between Days 3 and Day 5, at 38, 68 and 92 hours. Preliminary measurements suggested that the amount of curing compound applied on the specimen has an effect on the migration depth of the bacteria inoculated into the compound [14]. In order to see the effect of the dosage of curing compound, different ratios of liquid compounds were applied on the groups of the specimens. 0.3 v/v % (volume of compound/volume of specimen) compound was applied into the specimens in group I., 0.15 v/v % into group II. and 0.075 v/v % in group III. In order to test the ‘effect of equivalent volume’, 0.075 v/v % of curing compound, then 0.225 v/v % of distilled water was applied into the specimens in group IV. The control specimen was treated with non-inoculated compound. Dosage of the compound and of distilled water during the treatment is shown in Table 1.

### 3.4 Printing on agar plates for evaluation of the elevation height

Presence and type of a certain bacteria on different carriers can be determined by placing small samples (e.g. drops of liquid, part of the carrier by printing) on selective agar plates, since after incubation colonies grow on the surface of the agar [15]. Our idea was to enlarge size of the agar plate, so that whole surfaces of treated specimens could be printed on them. And, since distribution of the growing colonies of the bacteria within the print is identical to the distribution of the bacteria inside the porous matrix, we could evaluate how far bacteria migrated during the capillary absorption process. The specimens were printed onto the agar right after the end of the capillary absorption process, thus at the equilibrium point of the capillary absorption. All the cylinders were rolled over on the top of the solid agar, thus all of their surface area (except the tops

**Tab. 1.** Amount of biomineralizing compound applied on the specimens. In buckets the amount of additional distilled water.

Groups of the specimens	Mark of the specimens	14 h (ml)	38 h (ml)	68 h (ml)	92 h (ml)	Total amount per specimen (ml)
I.	4, 5, 8	30.72	23.04	23.04	15.36	92.16
II.	1, 3, 10	15.36	11.52	11.52	7.68	46.08
III.	9, 11, 12	7.68	5.76	5.76	3.84	23.04
IV.	2, 6, 7	7.68 (+23.04)	5.76 (+15.36)	5.76 (+15.36)	3.84 (+11.52)	23.04 (+69.12)
C	Control	(30.72)	(23.04)	(23.04)	(15.36)	(92.16)

and bottoms) were printed down. Edges and sides of the prints were marked with pins, as it can be seen in Fig. 2. At the end of the printing, containers were covered in a way that a small amount of air inlet was possible (half-sterile conditions). After 24 hours of incubation at  $24.5 \pm 1$  °C, colonies of *Bacillus cereus* grow on the agar. Then rulers were placed next to the sides of the prints on the agar, and all the containers were photographed from above, parallel to the surface of the agar.

Elevation heights of the bacteria due to the capillary elevation of the compound were evaluated with help of images taken of the containers. Elevation height was calculated as mean value of 10 measured distances of the farthest homogenous colonies along a vertical line from the bottom of the prints – see Fig. 3. Colonies grown dispersed above the border are considered to be error of the measurement.

Since it was suggested, that velocity of capillary elevation affects the migration height, the specimens were sorted according to the result of the capillary elevation tests. This way four groups were established with three specimens in each. Those specimens were classed into the first group, in which the instantaneous velocity of capillary elevation was the highest at every height. The second group consisted of the specimens with the second highest instantaneous velocities and in the same way with groups III. and IV. This way the affection of the migration height of bacteria by the differences in the absorbing behavior of the specimens was eliminated.

Due to the different density, surface tension and contact angle of the compound compared to the water, capillary absorption behaviors of the water and the compound are different. Still, it is reasonable to compare the absorption behavior of the two liquids, since they behave very similarly. Moreover, the water-absorption test can be performed on the same material as a preliminary test without causing permanent changes to the stone before the biomineralizing treatment. Thus, the above mentioned differences were neglected.

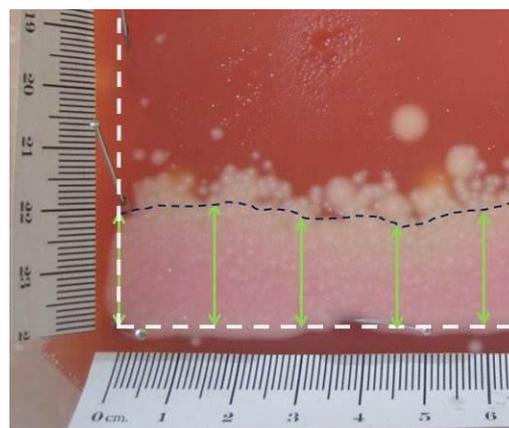
## 4 Results and evaluation

### 4.1 Mass properties of the stone material

Real density of the limestone was measured to be  $2.69 \pm 2.1$  (%)  $\text{g}/\text{cm}^3$ . Calculated mass properties of the specimens and  $w$  values are detailed in Table 2. Water-contents were



**Fig. 2.** Colonies of *Bacillus cereus* growing on the agar plate



**Fig. 3.** Evaluation of the elevation height: height of elevation (green), border of homogenous colonies (blue pecked line) and contour of the print (white pecked line)

found to follow the predicted order  $w_{h,max} < w_t < w_c$ .

#### 4.2 Capillary absorption test

Capillary elevation reached the top of the specimens in 390–540 seconds, and mass of the absorbed water was recorded until 660 seconds. In Eq. 1, according to velocity functions in [12] and Eq. 5, functions  $h(t)$  were found to be the best fitting on the measured ( $t; h$ ) points in a form of  $h(t) = \sqrt{c} \cdot \sqrt{t}$  where  $\sqrt{c}$  is a constant dependent on the type of the material of the specimen, calculated to range from 31.23 to 34.8. The maximum rate of evaporation ( $r_{e,max}$ ) was measured to be  $0.03 \pm 10\% \frac{g}{\min \cdot h_{max}}$ . With help of the  $r_{e,max}$  and the  $h(t)$  function, weight loss ( $m_{evap}(t)$ ) due to evaporation during the capillary elevation was calculated from Eq. 1 to be 0.14–0.156 g. This amount is less, than 1 m/m% (0.56–0.63 m/m%) of the absorbed amount of water when the elevation reached the top of the specimen ( $h_{max}$ ). Thus this loss of mass was neglected in the calculations of the capillary absorption.

However, mass of the water attached to the bottom of the specimen was measured to be significant,  $1.15 \pm 0.05$  grams (a layer of water with thickness of about 1 mm). With these constant values the volumes of the absorbed water were corrected, respectively. Evaluating the videos it was clear, that sinking of the water level in the bowl was parallel to the sinking of the specimen, thus uplifting effect of the water was also negligible.

Capillary absorption coefficients ( $k$ ) and capillary elevation coefficients ( $e$ ) of the specimens can be seen in Table 2.

It was found, that  $u_f$  values correlates best to the  $w_{h,max}$  values, in 99.78 to 103.91 %, respectively. This suggests, that  $u_f$  values correspond to the amount of water moving constantly upwards inside the porous matrix during the capillary elevation, until the front of the elevation reaches the top of the specimen. The measured and predicted relation  $w_{h,max} < w_t < w_c$  shows, that significant amount of water is absorbed after the elevation reaches the top, which is the result of the saturation of smaller capillary pores. Neglecting evaporation (or protecting the specimen against evaporation by e.g. wrapping its outer sides)  $w_t$  trends to get closer and closer to  $w_c$  after a long period of time.

#### 4.3 Printing on agar plates and evaluation of the elevation height

After 24 hours of incubation, bacterial colonies grown on all agar plates (Fig 3). As it was expected, the colonies were found only at the bottom part of the prints of the specimens. During the half-sterile incubation, microbes different from *Bacillus cereus* grew on some of the agar plates, too. Thus some of the prints were not appraisable. Therefore two results were obtained from group I, three from group II, one from group III, and two from group IV. No bacteria colonies of *Bacillus cereus* were observed on the control agar plate.

Measured maximum mean elevation height of the bacteria due to the capillary absorption turned out to be dependent on the volume of the compound applied into the specimen. Result of the

evaluation of the elevation height and the amount of compound applied on the stone specimens can be seen in Fig 4.

These results shows, that increasing amount of the compound absorbed by the stone increases the elevation height of the bacteria, but in a decreasing ratio of  $h/Q$ , where  $h$  is the elevation height in mm, and  $Q$  is the quantity of the absorbed curing compound in ml. Moreover, it was found, that almost the same height of elevation can be achieved by applying the same amount of bacteria and water into the specimen, than amount of pure curing compound. Applying 7.68 ml curing compound and applying 7.68 ml curing compound and 23.04 ml distilled water into the specimens resulted in a higher elevation of the bacteria in the latter case. This suggests that elevation height or migration depth of the bacteria can be improved by substituting some amount of the curing compound with water. The possible reason of these phenomena is that bacteria attach to the pore walls during the capillary absorption. But attachment of the bacteria can be dependent on the quantity and bacteria-concentration of the curing liquid compound. Since the bacteria are transported by the flowing compound, if larger amount of flowing liquid, as well as lower bacteria-concentration is available inside the specimen, bacteria will be transported further. Moreover, if bacteria are present in a lower concentration in the compound, they less likely attach to each other, thus they will be transported higher or deeper into the stone.

Elevation height of the bacteria was also evaluated in connection with the velocity of the absorption. It was found, that the velocities belonging to the elevation heights of the bacteria were different in all the cases. This suggests that elevation height of the bacteria is independent from the velocity of the absorption. Within the four groups of the specimens, the velocity belonging to the maximum elevation height decreased with the increasing amount of treating compound, as it can be seen in Fig 5. This result further proves, that amount of the curing compound is an essential factor in the migration depth of the bacteria.

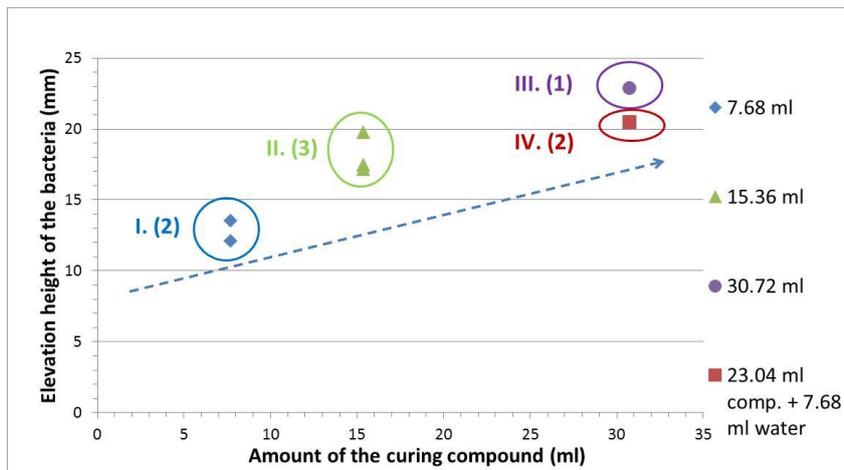
### 5 Conclusions

In the present study analysis of the capillary migration characteristics of *Bacillus cereus* present in a curing compound were evaluated in porous limestone of Sós-kút. For this purpose referred formulas, as well as results of two newly designed measuring methods were evaluated, and their results were combined. With help of the first, the printing-on-agar technique elevation height of the bacteria was determined. With help of the second method combined capillary absorption and capillary elevation characteristics of the porous limestone were specified.

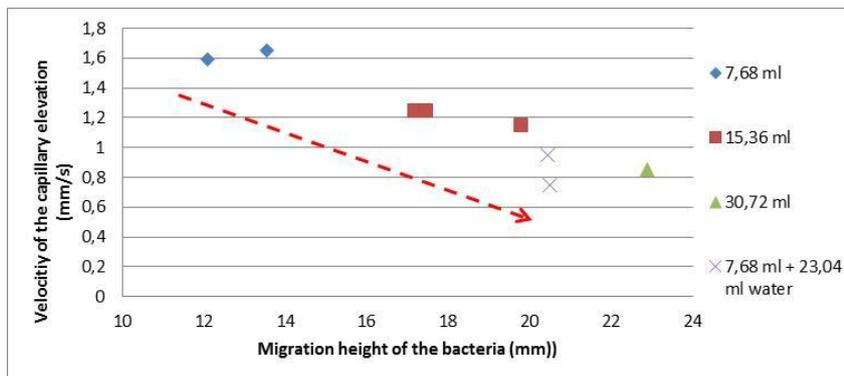
Both the printing-on-agar technique and the combined capillary absorption and capillary elevation test turned out to be efficient methods for the evaluation of the migration height of the bacteria. As first result of the experiments, relation between the capillary absorption ( $k$ ) and capillary elevation coefficients ( $e$ ) was validated, and their values were calculated to be  $k = 1.08 \pm 0.06 \frac{kg}{m^2 \cdot \sqrt{s}}$  and  $e = 0.0041 \pm 0.00 \frac{m}{\sqrt{s}}$ . With help

**Tab. 2.** Mass properties and capillary absorption properties of the specimens

Mark of the specimen	Apparent density	Constant water content / Apparent porosity ( $w_c$ )	Total porosity	Water-content at $h_{max}$ ( $w_{h,max}$ )	Capillary absorption coefficient ( $k$ or $A$ )	Capillary elevation coefficient ( $e$ )	$\frac{u_f}{e \cdot \rho_w}$	$w_t$
-	$\frac{g}{cm^3}$	$\frac{m^3}{m^3}$	$\frac{m^3}{m^3}$	$\frac{m^3}{m^3}$	$\frac{kg}{m^2 \cdot \sqrt{s}}$	$\frac{m}{\sqrt{s}}$	$\frac{m^3}{m^3}$	$\frac{m^3}{m^3}$
1	1,563	0,333	0,420	0,269	1,14	0,0043	0,268	0,292
2	1,563	0,337	0,420	0,247	0,99	0,0039	0,252	0,270
3	1,564	0,336	0,419	0,280	1,15	0,0041	0,279	0,306
4	1,567	0,322	0,418	0,272	1,11	0,0041	0,272	0,289
5	1,563	0,318	0,420	0,261	1,00	0,0039	0,260	0,278
6	1,562	0,326	0,420	0,271	1,11	0,0041	0,271	0,298
7	1,562	0,321	0,420	0,266	1,08	0,0041	0,265	0,283
8	1,566	0,323	0,419	0,258	1,18	0,0044	0,268	0,280
9	1,580	0,321	0,414	0,247	1,09	0,0043	0,255	0,278
10	1,560	0,324	0,421	0,245	1,05	0,0042	0,251	0,275
11	1,544	0,319	0,427	0,254	1,10	0,0043	0,253	0,277
12	1,564	0,320	0,419	0,256	1,01	0,0039	0,261	0,277
13	1,563	0,324	0,420	0,255	0,99	0,0039	0,255	0,275
<b>Mean</b>	<b>1.56</b>	<b>0.325</b>	<b>0.420</b>	<b>0.260</b>	<b>1.08</b>	<b>0.0041</b>	<b>0.262</b>	<b>0.283</b>
<b>±stand. dev.</b>	<b>±0.01</b>	<b>±0.006</b>	<b>±0.00</b>	<b>±0.01</b>	<b>±0.06</b>	<b>±0.00</b>	<b>±0.009</b>	<b>±0.01</b>



**Fig. 4.** Effect of the amount of the curing compound on the migration height of the bacteria. Number of the groups are with roman letters, after them the number of valid measurements in brackets.



**Fig. 5.** Relationship between the migration height of bacteria, the velocity of the water absorption and the amount of curing compound.

of these coefficients, exact physical meaning of the free water-content was found to be the water content at the moment the front of the elevation reaches the top of the specimen. Its value was measured to be  $0.262 \pm 0.009$ . Combined results of the two tests showed, that elevation height of the bacteria can be enhanced upon application of more liquid curing compound. Elevation heights of the bacteria were recorded to be 12.1 - 22.9 mm, dependent on the amount of applied curing compound. It was also found, that the amount of the curing compound can be partly substituted with the post-addition of water without significant decrease of the migration height. This result should be considered in case of in-situ biomineralizing treatments, where enhanced effective depth of the treatment is desirable.

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