Preliminary research for long lasting self-healing effect of 
bacteria-based concrete with lightweight aggregates

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Abstract. A typical phenomenon in concrete structures is crack formation. Shrinkage cracks may appear in early stage of concrete. In late age cracks may appear due to process of serviceability and external weather conditions. Larger cracks interfere structural integrity, micro-cracks may result in durability problems as increase of water absorption, which results in concrete degradation and reinforcement corrosion. Manual maintenance of the concrete structure is costly and labour consuming; therefore, functionality of self-healing phenomenon of concrete structures has been inspected.

In this study self-healing phenomenon of bacteria-based concrete has been investigated. Calcium lactate and yeast medium with spore-forming alkaliphilic bacteria Bacillus Lentus have been chosen. Bacillus Lentus spores with medium were integrated (by using vacuum technique) into expanded clay granules (LECA) with a size of 2-4 mm. Direct addition of bacteria (without immobilization) in concrete limits the survival of bacteria from 1 to 2 months. In this study, to increase viability of bacteria at high pH media in concrete and thus the time-related functionality of self-healing agents, bacteria were immobilized in LECA.

The mechanism of crack healing in bacteria-based concrete presumably occurs through metabolic conversion of calcium lactate to calcium carbonate. The influence of bacteria on the mechanical and physical properties of samples was estimated after 28 days and after 6 years. From the results obtained, it can be concluded that concrete samples with Bacillus Lentus showed an increase of 22% in compressive strength compared to the reference sample.

The main task of this scientific research is to identify, if self-healing phenomenon happens for the samples, which are 28 days old and for samples which are 6 years old. Results from the optical investigation showed that self-healing phenomenon (after 14 days of healing) has occurred for all samples and cracks in concrete were partly closed with calcium carbonate.

1. Introduction
Concrete is the most essential and popular material for building structures, due to high serviceability, compressive strength, and availability of raw materials.1 However, the mechanical strength and durability of concrete structures can be seriously deteriorated by micro or macro-cracks.2 Typically, penetrations of water and chloride through the cracks drastically affect the durability of concrete structures and cause the corrosion of steel bars, which consequently lead to failure issues.3 Therefore, it is vital to heal or repair these cracks to maintain the serviceability of concrete structures.4 Cracks can be repaired manually in rare situations, because most of these repairing operations are restricted by accessibility, location, cost, and environment problems.5 Hence, self-healing of cracks has become a
necessity for concrete structures, and attracts rising attention of researchers. On the other hand, the autogenous repairing phenomenon of concrete structures has been observed in natural environment for many years. It is accomplished by formation of calcium carbonate and continuous hydration of anhydrous cementitious materials, when water and carbon dioxide are available. However, without further modification or improvement, the efficiency of those healing activities is relatively low. Therefore, various strategies have been developed to improve self-healing behavior, typically though inserting kinds of additions into the cementitious materials, such as crystalline admixture, polymers, hollow fiber, mineral admixtures, encapsulations, nanoparticles, and microorganisms.

In this study, in order to prolong the service life of concrete structures the self-healing concrete with alkaliphilic bacteria from the genus of Bacillus and organic compounds was developed. Production of the self-healing concrete mix does not differ from the ordinary cement mix production of the lightweight concrete contained cement, sand, lightweight aggregates and water. Before mixing lightweight aggregates were impregnated with the bacteria Bacillus Lentus and the organic mineral compound (calcium lactate and yeast) in order to encapsulate self-healing agent and prevent it from the degradation at high pH of concrete. Bacteria spores, calcium lactate and yeast extract were dissolved in water and solution was used for impregnation of bacteria into the pores of the expanded clay granules (LECA) by using vacuum treatment. After impregnation and drying the bacteria spores localize inside of the lightweight aggregates, but they are not active. They become active when cracking of concrete appears and presence of water and oxygen is possible. After activation the bacteria start to degrade the calcium lactate and produce calcium carbonate that is sealing the open cracks.

The main goal of this research is to estimate the Bacillus Lentus activity after pre-cracking of 28 days and 6 years old lightweight concrete samples. The crack self-sealing phenomenon was estimated by optical microscope. Physical and mechanical properties of bacteria-based concrete were determined.

2. Materials and methods
2.1. Preparation of the healing agent
The bacteria-based healing agent consisted of spores derived from alkaliphilic bacteria of the genus Bacillus and organic mineral compounds. Calcium lactate and yeast medium with spore-forming alkaliphilic bacteria Bacillus Lentus have been chosen to produce bacteria-based concrete. Calcium lactate is a salt that consists of two lactate anions for each calcium cation (Ca2+). It is prepared commercially by the neutralization of lactic acid with calcium carbonate or calcium hydroxide. Calcium lactate is also found in daily dietary supplements as a source of calcium. It is also available in various hydrate forms, where calcium lactate pentahydrate is the most common.

The healing agent was incorporated in lightweight expanded clay aggregate (LECA) via solution absorption during 24 hours, 72 hours and an impregnation under vacuum with calcium lactate, yeast extract and bacteria spores solution.

Two solutions were prepared for impregnation into LECA. Firstly, water with calcium lactate (1.5% added of total liquid medium) and yeast (0.03% of total liquid medium). Calcium lactate and yeast extract were heated at 40 °C for about 1 h to dissolve in water. Then the same steps were taken as for the LECA impregnation with water and bacteria spores.
Maximal amount of bacteria and calcium lactate and yeast extract medium which is possible to impregnate in the LECA was calculated.

2.1.1. Absorption test for 24 and 72 hours. For 24 hour and 72 hour absorption test 500g of LECA was taken and immersed in 3 solutions. To avoid aggregates flowing to the top, LECA was put inside the fabric cloth and pushed to the bottom of the container with 0.5mm sieve. After 24 and 72 hours, LECA was taken out of the solutions, dried with wet cloth and mass was measured. Then LECA was placed in an oven at 20°C until it reaches a constant weight.
After 24 hour absorption test it was concluded, that solution uptake for LECA is in a range of 18-22%. Calcium lactate amount inside the LECA aggregates ranges from 0.27-0.33%, yeast extract ranges from 0.0054-0.0066%, bacteria spores 2x10^8.

After 72 hour absorption test it was concluded, that solution uptake for LECA is in a range of 24-25%. Calcium lactate amount inside the LECA aggregates ranges from 0.36-0.375%, yeast extract ranges from 0.0072-0.0075%, bacteria spores 2x10^8.

2.1.2. Impregnation under vacuum. Initially LECA was dried at 20°C for 24 h to remove moisture content. The weighted amount (500 g) of the LECA was added in the vacuum desiccator at −0.95 bar by employing a suction pump. The LECA was spread in between two metallic sieves with a mesh size 0.125 mm to enhance surface contact between the aggregate and the water. Liquid medium was injected from the top through a funnel to flow under gravity past the LECA through sieves. The medium was kept injected inside the desiccator until the porous aggregate was fully submerged. The top sieve is installed to prevent LECA floating over the water surface when a vacuum is applied. The bottom sieve is installed to prevent settling of the heavier LECA mix when the vacuum is removed. The vacuum was applied for 10 min. The medium is assumed to keep impregnation into LECA as long as air bubbles keep appearing under vacuum.

After impregnation test it was concluded, that solution uptake for LECA is in a range of 55-57%. Calcium lactate amount inside the LECA aggregates ranges from 0.825-0.855%, yeast extract ranges from 0.0165-0.0171%, bacteria spores 2x10^8.

Following the impregnation, the LECA were dried for approximately 5-6 days at standard temperature (20±2°C), until a constant weight was achieved. During drying procedure, the spores can remain in the dormant state, since the pH of the environment is not adequately high for them to activate and start germinating. It was found, that after drying, the impregnated LECA did not increase their initial weight (only 0.05%) due to the addition healing agent.

![Calcium lactate is crystallized after five days of drying at 37 °C.](image)

As from the Figure 1 it is seen, after drying of LECA calcium lactate crystallized on the surface of LECA. In order to ensure, that the crystallized medium do not have negative aspect on the hardened concrete and their mechanical properties, it was concluded to prepare cubes 10x10x10 cm and test concrete compressive strength.

2.1.3. Samples for mechanical properties. The aim of this study was to determine how the self-healing medium, such as calcium lactate, yeast extract and bacterial spores, affect the compressive strength of concrete matrix. Sample compositions are given in Table 1.

Main ingredients for production of the bacteria-based concrete were: Portland Cement CEM I 42.5N, locally available sand (combination of 2 fractions and quartz powder), lightweight expanded clay
aggregates and water. In this study lightweight expanded clay aggregates (LECA) from Ltd “Fibo” were used with size from 2 to 4 mm, density - 250 kg/m³, porosity – 82%.

**Table 1. Sample compositions.**

| Components                                         | Weight, g | REF | SH1 | SH2 | SH3 |
|----------------------------------------------------|-----------|-----|-----|-----|-----|
| Lightweight expanded clay aggregates               | 292.0     | x   |     |     |     |
| Lightweight expanded clay aggregates (bacteria)    | 292.0     |     | x   |     |     |
| Lightweight expanded clay aggregates (calcium lactate, yeast extract) | 292.0 |     |     | x   |     |
| Lightweight expanded clay aggregates (medium and bacteria) | 292.0 |     |     |     | x   |
| 0.5-1 mm sand                                      | 397.0     |     |     |     |     |
| 0.25-0.5 mm sand                                   | 346.0     |     |     |     |     |
| 0.125-0.25 sand                                    | 186.0     |     |     |     |     |
| CEM I 42.5 N                                       | 384.0     |     |     |     |     |
| Water                                              | 192.0     |     |     |     |     |
| W/C                                                | 0.5       |     |     |     |     |

Compressive strength of bacteria-based lightweight concrete was determined according to LVS EN 1015-11 using specimens with dimensions 10x10x10 cm. The density of samples was measured in accordance with EN 1097-7. An optical microscope “AmScope” was used to evaluate self-healing of the concrete cracks. Total porosity for LECA was obtained from specific gravity obtained by Le Chatelier flask (ASTM C188).

**2.1.4. Samples for self-healing test.** LECA was impregnated with self-healing substances which consisted of bacterial medium - calcium lactate, yeast extract and bacterial spores. Impregnation process consisted is described before in 2.1.3.

For self-healing testing ability, SH3 samples with dimensions of 4x4x16 cm were prepared (Table 1). After filling the concrete mass into moulds, the samples were vibrated for 3 min on the vibration table (40 Hz) to ensure that the concrete mass was evenly compacted. The samples were hardened in the moulds for 3 days, covered with polyethylene film. Afterwards the specimens were cured in water for 2 days, then in final stage - the curing continued in a chamber with air temperature of + 20 ± 5 °C and relative humidity of air ≥95%.

**2.2. Cracking of mortar prisms**

After removing the samples from the curing chamber, the wet areas were wiped with a dry cloth. The dimensions of the samples were determined using the gauge.

Three-point-bending (with a span of 100 mm) was used for the crack induction on 28-days-old samples and 6-years-old samples. A single crack was created in each specimen. A vertical load was applied in the middle of the specimens until the formation a stable crack. While loading, the crack opening increased constantly by 0.0005 mm/s until it reached approximately 0.4 mm. The crack width was monitored via two Linear Variable Differential Transducers attached on the front and the back of the side of the specimens. When the crack width reached 0.4 mm the specimens were slowly unloaded. After unloading, the crack width reduced to approximately 0.27-0.36 mm. The depth of the crack was not monitored during bending, but it was observed through microscopic images.
2.3. Healing treatment
Following the crack creation specimens were placed horizontally in a plastic container filled with tap water for crack healing. Samples were completely immersed in water while placed on the spacers. The container was kept open to the atmosphere at standard room temperature (20±2 °C) with (60±10) % RH. Samples at the age of 28 days and 6-years-old samples were left to heal for 14 days. Extra water was added, to keep a constant liquid-to-solid ratio.

2.4. Crack inspection
Crack inspection was used to primary evaluate the sealing efficiency of the bio-based healing agent. The inspection was conducted in two steps, i.e. right after crack creation and after healing treatment.

3. Results
3.1. Mechanical and physical properties of the concrete matrix
The density of the samples ranged from 1736 to 1777 kg/m³. Scientific literature describes that calcium lactate, a bacterial nutrient, has a positive effect on the compressive strength of concrete, improving it by 10%. In addition to calcium lactate, a yeast extract that functions as a bacterial nutritional supplement was added. According to literature yeast extract may significantly reduce the compressive strength of concrete.

Table 2. Physical and mechanical properties of the 28-days old 10x10x10 cm samples.

| No | Composition | W/C ratio | Age of concrete | Density, kg/m³ | Compressive strength, MPa | Concrete class |
|----|-------------|-----------|----------------|----------------|---------------------------|---------------|
| 1. | REF         | 0.5       | 28 days        | 1736           | 21                        | C20/25        |
| 2. | SH1         | 0.5       | 28 days        | 1767           | 22                        | C20/25        |
| 3. | SH2         | 0.5       | 28 days        | 1777           | 27                        | C20/25        |

After 28 days of hardening, the concrete samples were tested. The compressive strength of the REF sample was 21 MPa, the sample with medium impregnated in LECA (SH2) - 27 MPa and the sample with Bacillus Lentus bacteria spores impregnated in LECA (SH1) - 22 MPa.

From the results obtained, it can be concluded that both of the concrete samples with self-healing reagents showed better compressive strength results compared to the reference sample. The sample with bacterial nutrients impregnated in LECA showed 22% increase in compressive strength compared to the reference sample.
It is concluded that adding calcium lactate (1.5% of the total liquid medium) and a small amount of yeast extract (0.03% of total liquid medium), compressive strength increases and the medium gives a positive effect on bacterial growth and development.

3.2. Results from the self-healing test

The specimens were loaded until the crack opening reached 0.4 mm. However after unloading the crack width was < 0.3 mm. Specimens (28-days old and 6-years old) were submerged in water for 14 days for self-healing to occur.

![Figure 3](image1.png)

**Figure 3.** 28-days old sample after cracking (A), self-healing of the cracks for 28-days old sample after 2 weeks in water (B).

![Figure 4](image2.png) ![Figure 5](image3.png)

**Figure 4.** Calcium carbonate precipitation in the cracks of 28-days old SH3 sample. **Figure 5.** Calcium carbonate precipitation in the cracks of 28-days old SH3 sample.

In Figure 4 and 5 cracks in the concrete matrix at 40x magnification using an optical microscope are shown. White crystalline material precipitates on both sides of the crack. The cracks are not completely healed, but precipitation is observed throughout the concrete matrix which had direct water contact. Crack size varies up to 0.3 mm.

The self-healing results from the 28-day-old samples were observed after 2 weeks (Figure 4 and Figure 5). It is concluded, that self-healing processes are happening and this medium and Bacillus Lentus are successful in ensuring self-healing ability of concrete. In the scientific literature it is mentioned, that the crack healing process with bacteria can last from 2 weeks to several years.14

In the beginning of the experiment, it was considered, that all the healing agent inside the LECA will be released when LECA will be intersected by crack and will convert to CaCO3. In reality, only part of the healing agent is released, while the rest remains in the lightweight aggregate (it was observed in the samples after 14 days of healing).
Autogenous healing processes, that occur during the healing treatment in water for 28-days old samples were not considered.

6-years old samples were pre-cracked and tested on self-healing ability. All the conditions for the test experiment were ensured the same as for the 28-days old samples in year 2013. Samples were loaded with 50kN large force, crack openings were up to 0.3 mm. In Figure 6 the self-healing process has happened in the range of 60% to 90%, depending on the size of cracks in the concrete matrix. Precipitation of calcium carbonate was determined.

Figure 6. Calcium carbonate precipitation after 2 weeks for 6-years old SH3 sample.

It is possible to exclude the fact that these calcium carbonate precipitations for 6-years old samples are from hydration processes (Figure 6), because concrete samples were made and maintained under appropriate conditions to allow complete hydration of the concrete, and the water cement ratio (0.5) of the samples was not high.

It can be stated, that crack closure percentage increases for the specimens immersed in water for longer time period (specimens were monitored each day during time period of 14 days). During the monitoring process of the cracks it was concluded, that the narrower the crack the more efficient is the sealing.

4. Conclusions
It was concluded, that reactions, that take place inside the cracks are complex and depend on several factors, such as local crack width, the presence of oxygen, the duration of healing treatment etc. Experimental approaches might overestimate the crack closure due to the hypothesis that the crack filling is constant along the crack length, but it is not.

It is concluded that addition of calcium lactate (15% of total liquid medium) and a small amount of yeast extract (0.03% of total liquid medium) increase the compressive strength and the medium gives a positive effect on bacterial growth and development. Addition of calcium lactate (15% of total liquid medium) to a concrete mix improved compressive strength by 22%. The yeast extract in low dose (0.03% of total liquid medium) had no negative effect on the compressive strength of the concrete. Addition of bacterial spores to the concrete did not have a negative effect on compressive strength, compared to the reference samples, the compressive strength increased by 2.31%.

After 2 weeks in the water for 28-days samples cracks were partially healed, in the range of 10% to 90%, depending on the size of the crack in the concrete matrix.

Bacteria immobilization in LECA ensures bacteria viability after many years. After 2 weeks in water for 6-years-old samples crack healing was observed, in the range of 60% to 90%, depending on the size of crack. We can conclude, that Bacillus Lentus spores have survived 6 years old dry period and calcium carbonate precipitation in the cracks is obvious. Self-healing of 6-years old samples have happened.
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