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The effect of temperature on bacterial self-healing processes in building materials

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Abstract. This paper is focused on the bacterial induced calcitation for the crack healing. The bacteria applied for this purpose are from group which is adapted for growth in the high pH environment as a concrete in hydration phase and their metabolic activity leads to create of calcite. In this study, three different bacteria strains (*Sporosarcina pasteurii, Bacillus cohnii, Bacillus pseudofirmus)* were applied and the influence of various temperatures on their microbial properties was investigated. Our previous experiment indicated that one of the applied bacterial strain in spores form (*Bacillus pseudofirmus*) are able to survive the temperatures in the range from −20 °C to 140 °C. The experiment described in this paper extends the previous study and determines the effect of different temperatures on the change in growth activity. In this experiment, bacterial activity was determined based on the change of absorbance in 640 nm by spectrophotometric measurements. The experiment was performed at optimal temperature (30 °C) and lower temperature (10 °C) and it used suitable broth for calcitation. The results showed that the beginning of metabolic activity was shifted by 40 to 50 hours. Only *Bacillus cohnii* showed different results because its metabolic activity was nearly zero at 10 °C.

1. Introduction

Concrete is the most used construction material worldwide. Degradation of this material is known and described phenomena. The main degradation processes are caused by pollutants present in the atmosphere such as oxides of nitrogen, carbon, sulphur, acids and many other compounds. These processes are known as atmospheric corrosion or chemical degradation. Nevertheless, even though concrete structures generally undergo a number of degradation processes, they are designed to maintain their service life for more than 50 years [1].

These mentioned problems lead to higher porosity and subsequent to increase concentration of aggressive substances in pore system. The aforementioned compounds mainly react with free calcium ions which results in a decrease in pH, thus durability of the material is endangered. Nowadays, many types of repair systems are available. However, these epoxy-based fillers or silane-based water repellent systems may have only short-term effectiveness or a negative impact on the environment. Today it focuses more on instant repair or self-healing of cracks may extend the service-life of concrete structures [2].

Bacteria can be found everywhere and some of them are resistant enough to survive in various harsh conditions. The self-healing of concrete can be caused by some autogenic processes or induce by bacteria. In both cases, the main reactions lead to creation of new crystals of calcium carbonate

($CaCO_3$). Microbially induced calcification (MIC) is a novel innovative alternative method for protection of concrete construction against change of porosity. This phenomenon has been known for many years [3-5]. Ramakrishnan published a pilot study which focused on bioremediation as a treatment of cracked concrete [6]. Some bacterial strains can induce a reaction between atmospheric carbon dioxide and calcium ions, see Equation 1. Other ones can use the hydrolysis of urea which is catalysed by urease. As a consequence, urea is degraded into carbonate and ammonium, resulting in an increase in the pH and carbonate concentration in the bacterial environment [7], see Equation. 2.

$$H_2O + CO_2 \rightarrow HCO_3^- + H^+$$
$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + H^+ + HCO_3^- \rightarrow CaCO_3 + H_2O + CO_2 \quad (1)$$

or with urea

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-}$$
$$CO_3^{2-} + Ca^{2+} \leftrightarrow CaCO_3 \quad (2)$$

In this paper, the bacteria for microbially induced calcification were selected from groups which are adapted to colonization in a high pH environment and can produce calcium carbonate (*Sporosarcina pasteurii, Bacillus cohnii, Bacillus pseudofirmus). Sporosarcina pasteurii* is a gram-positive bacterium able to survive in highly alkaline environments (pH ~ 10) and has ability to secrete a copious amount of enzyme urease and use the second aforementioned equilibrium (Eq. 2). The group *Bacillus* includes facultative and obligate alkaliphilic species that grow well at pH values (higher than 9.0). Alkaliphilic *Bacillus* strains secrete large amounts of alkaline proteases with a high stability at elevated pH and temperature values.

This novel strategy for preventing crack formation seems to be the right direction to minimize repair costs of constructions. The bacteria which we mentioned above have great potential for self-healing due to their beneficial characteristics (pH resistance and ability to produce calcite). However, previous studies pointed out several problematic aspects which need to be solved e.g. the application of bacteria with non-reduction of material properties and bacteria survival in the first period of cement hydration. Further, the investigations must focus on the restart of the bacterial life cycle and determine the necessary conditions for successful bacterially induced calcification. The main question is the minimum time for water to be in pore system of material in specific conditions (T, pH, strain bacteria, source of calcium). This article follows the previous experiment which dealt with the survival of bacteria after freeze-thaw cycles between -20 °C to 5 °C [8]. The current study wants to determinate the necessary time to restart the bacterial life cycle in temperatures between 10 °C and 30 °C.

2. Materials and methods

The selected bacteria were obtained from Czech collection of microorganisms (CCM) or from Belgian Coordinated Collections of Micro-organisms (BCCM). The access number of strains were: *Sporosarcina pasteurii CCM 2056, Bacillus cohnii CCM 4369, Bacillus pseudofirmus* (LMG 17944). The used culture media are described in Table 1. The media were prepared based on the prescriptions from CCM or BCCM.The medium 53 contain following compounds: Lab-Lemco' beef extract 1 g/l, yeast extract 2 g/l, peptone 5 g/l, NaCl 5 g/l, agar 15 g/l and the pH 9.7 was achieved by Na-sesquicarbonate solution after sterilization in an autoclave.

Table 1. The correlation between bacterial strain, media and optimal temperature.

Strain	Optimal temperature [°C]	Growth medium
Bacillus cohnii	37	53
Sporosarcina pasteurii	30	B13
Bacillus pseudofirmus	30	253

The Na-sesquicarbonate solution consisted of NaHCO3 4.2 g, Na_2CO_3 anhydrous 5.3 g in distilled water 100 ml, the sterilization was performed by filtration. The medium 253 was prepared based on the medium 53 only with 0.5% NaCl. The medium B13 contained following compounds: Lab-Lemco' beef extract 1 g/l, yeast extract 2 g/l, peptone 5 g, NaCl 5 g/l, agar 15 g/l, urea 20 g/l. The pH value was adjusted to 7.4 after sterilization by Na-sesquicarbonate solution described above.

The bacterial inoculation for experiments were prepared by three time repeated follow cultivation for starting the maximal growth rate in optimal condition for seven days. Further, this inoculum was applied in a ratio 1 ml of the inoculum to 100 ml of the fresh media for the biological oxygen demand (BOD) experiments and 1 ml of the inoculum to 50 ml of the fresh media for the ELISA experiments.

2.1. ELISA measurement

The amount of bacteria cells or multiplication were measured by change absorbance in 630 nm. The values were recorded every hour in the daytime for the first 5 days, then the data collection frequency decreased to 5 times in the daytime. This obtained data was used for determinate of growth curve. The whole experiment was performed for 14 days.

2.2. BOD experiments

The Biological Oxygen Demand (BOD) method is based on the measurements of the amount of oxygen by consumed by aerobic microorganisms. In our study, this method was used for the determination of metabolic activity of the investigated bacteria. The BOD values are most commonly expressed in milligrams of oxygen consumed per litter of a sample during 5 days of incubation at 20 °C. The BOD device is standardly calibrated to temperature around 25 °C. For this reason, the BOD method must have been firstly tested on a known bacterial strain in other than the calibration temperature in order to determine its applicability in our experiment. The bottles for BOD analysis were filled with 100 ml of fresh media respectively tempered to the selected temperature. Subsequently, the bacterial inoculums in a volume of 2 ml were added respectively. The bottles were closed and placed into the cultivation temperature. The measurements were continuous (i. e. once every 0.5 h for 5 days).

3. Results

The influence of different temperatures (10 °C and 30 °C) on the bacterial growth was determined as a change of optical density by ELISA. The bacteria *Bacillus pseudofirmus* (Figure 1) had a longer log phase at the lower temperature but the optical density was similar in 145 hours of experiment.

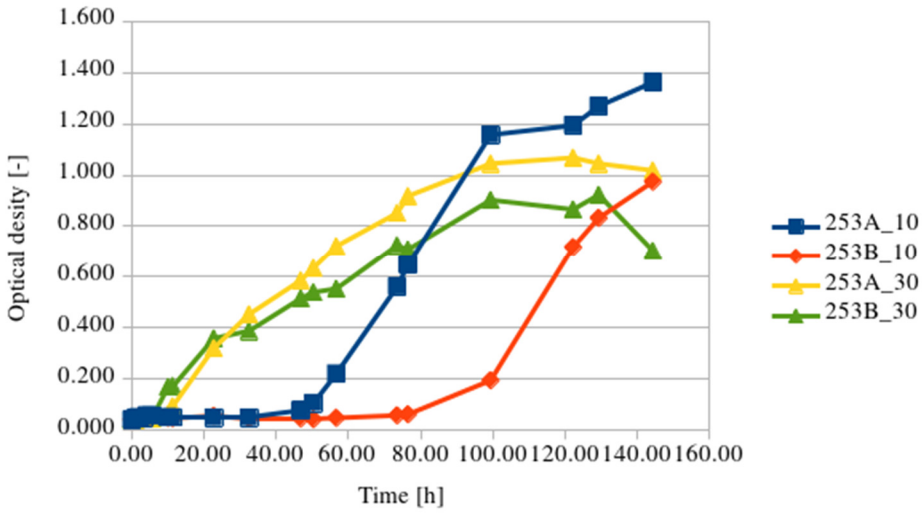

Figure 1. The growth curve for *Bacillus pseudofirmus*, the number after label is the used temperature for experiment, 10 °C or 30 °C.

The ending of the log phase of the *Bacillus pseudofirmus* cultivation at 10 °C was between 45 – 76.5 hours. The log phase of the *Bacillus pseudofirmus* cultivation at 30 °C was shorthened to 10.5 hours.

For the results for ELISA test of bacteria *Sporosarcina pasteuri* (Figure 2). The log phase was shifted as a results for *Bacillus pseudofirmus.* The bacteria growth has started in range 32.5 to 50.6 hours in 10°C and in 22.5 hours in the 30 °C experiment. The limited supply of nutrients was detected in 140 hours. The metabolic activity measured by BOD started during the 1st hour for the 10 °C cultivation and during 30 minutes for the 30 °C cultivation. This activity was stable throughout the whole experiment.

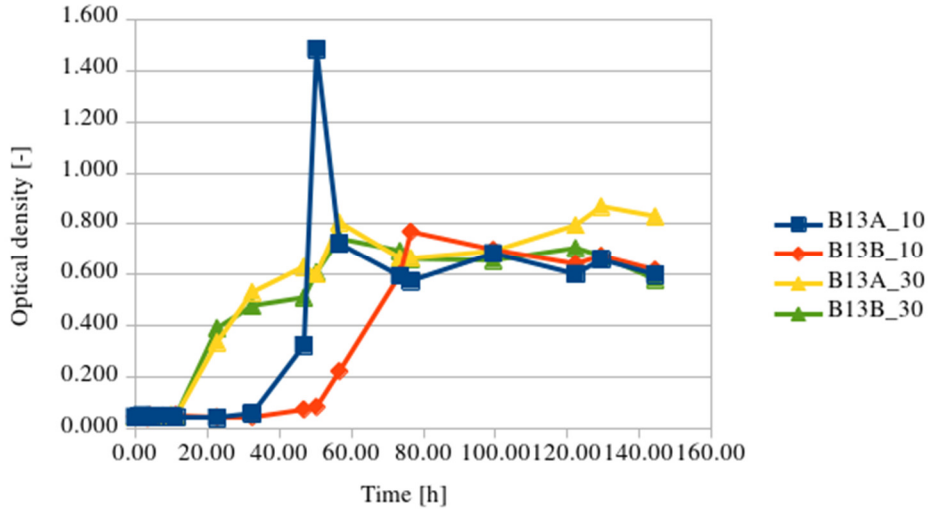

Figure 2. The growth curve for Sporosarcina pasteurii, the number after label is the used temperature for experiment, 10 °C or 30 °C.

The multiplication of *Bacillus cohnii* can be seen in Figure 3. This cultivation has different results from the other investigated bacteria. The log phase was the longest of all used bacterial strain The bacteria at 10 °C did not start the growth until 150 hours.. The cultivation at 30 °C showed very different results in comparison with the 10 °C results. The start of multiplication was between 32.5 and 122.5 hours.

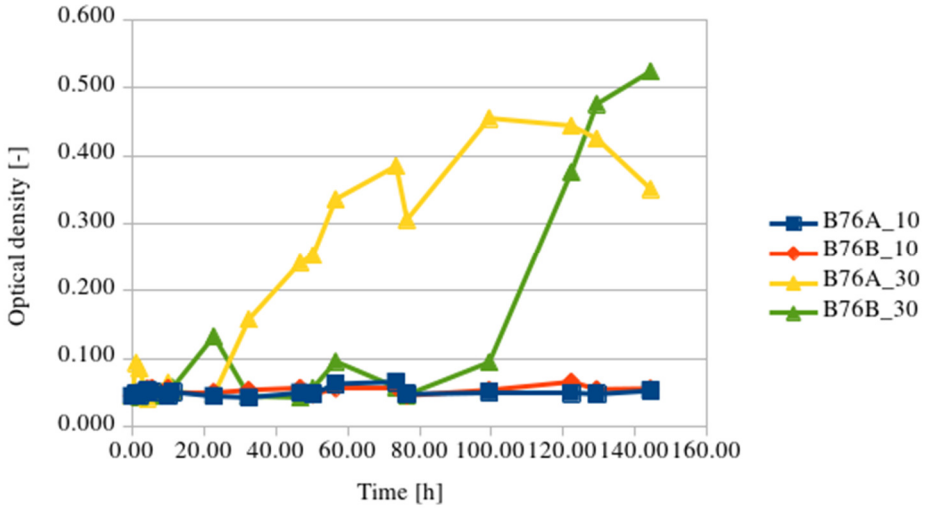

Figure 3. The growth curve for *Bacillus cohni*, the number after label is the used temperature for experiment, 10 °C or 30 °C

The metabolic activity measured by BOD for this temperature was lower but stable in the whole experiment. The metabolic activity measured by BOD system showed lower value, but they were stable for 140 hours. These results show that *Bacillus cohnii* have not stable growth characteristics and also our previous unpublished measurements indicated that the bacteria have very unstable growing activity in pH more than 8.5.

4. Conclusion

In this study, three strains of bacteria were studied and the possibility of their application for the bacterially induced calcification was determined. All of the selected bacteria are from the class *Bacilli*, two of them are from genus *Bacillus* and one from *Sporosarcina*. These bacteria are isolated from environment with high alkalinity therefore they have properties to survive during hydration of concrete. These three strains can survive at temperature under -5°C in the form of spores, and they further restart their metabolic activity when the conditions become optimal. The time required to the metabolic activity recovery depends on the water content, nutrient source and temperature. As it has been expected, the results of metabolic activity in lower temperatures (around 10 °C) showed that the recovery time is longer as it between 32.5 to 76.5 hours for *Sporosarcina pasteurii* and *Bacillus pseudofirmus*. The strain *Bacillus cohnii* did not recover multiplication in our experiment. The higher temperature is approaching their optimal temperature and their recovery was faster. It is around 20 hours. The strain *Bacillus cohnii* had longer period in one of the cases but the results indicated the strains instability in the high alkalinity conditions.In this study, the results showed that temperature around 10 °C is sufficient for the recovery of metabolic activity of two of the applied bacterial strains (*Sporosarcina pasteurii* and *Bacillus pseudofirmus).* but the time require for wetness material are longer. The temperatures closer to optimal showed better results as expected. However, maintaining high water content at this temperature in real environment is very problematic. The further studies should deal with protection of bacteria during hydration period and keep.

Acknowledgement

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