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Self-healing in cementitious composite containing bacteria and protective polymers at various temperatures

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Abstract. Autonomous sealing of cracks in concrete through bacteria-induced calcification has become a topic of great concern in the last two decades. This paper is focused on two main issues of the so-called bio-based self-healing concrete, i.e. protection of the bacterial spores embedded in the cementitious matrix and behavior of the material at low temperatures. The second aspect is particularly important as the impact of the conditions corresponding to real outside environment was rarely investigated before. An investigation of the influence of temperatures below the freezing point is a unique extension of the current state of the art. In the current study, as a form of protection, superabsorbent polymers (SAP) powder and 16 % polyvinyl alcohol (PVA) water solution are applied. The performed mechanical tests showed pronounced negative impact of the PVA addition on both tensile and compressive strength (a decrease of 56 % and 79 %, respectively), while the SAP negatively affected only the compressive strength (a drop of 30 %). In our study, the composite containing SAP reached even slightly higher tensile strength compared to the control (around 7 % increase). The healing action was observed on cracked cementitious composites beams at ideal (i.e. room) temperature, low temperature (10 °C), and after exposure to freeze cycles (–5 to 0 °C). After 28-day immersion in water at the ideal temperature, the series containing SAP and bacterial spores (BAC_SAP) showed the most pronounced healing – the value of the average maximum healed crack width (Δw_{max}) reached 219 µm. In the case of preliminary freeze cycling, the BAC_SAP also reached the highest values. At low temperatures, the positive impact of SAP seems to be inhibited as Δw_{max} is the highest in the control series. In all of the applied conditions, insufficient crack-sealing was detectable in the samples containing PVA. Thus, the SAP proved to be applicable for the protection of bacterial spores at ideal temperatures; however, more research concerning its mechanism in cementitious composite at lower temperatures is needed.

1. Introduction

1.1 State of the art

The reduction in the durability of concrete structures is closely related to the presence of cracks in their cover layer. Cracks accelerate the transport processes in the porous structure of concrete, thus making the material more susceptible to degradation (such as chloride corrosion, carbonation, etc.). Conveniently, concrete is known for its so-called autogenous crack sealing [1], in which the formed damages are gradually (completely, or at least partially) sealed. However, this complex phenomenon is influenced by a large

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number of factors, and it cannot be easily quantified. Thus, it is not possible to completely rely on this natural behavior and regular manual inspections and repairs of the cracked surfaces must be undertaken.

However, this natural ability of cementitious materials has inspired researchers worldwide to the idea of "self-healing" concretes – cementitious materials which could autonomously detect and repair its cracks. In the 19th century, the ability of certain microorganisms, specifically bacteria, to produce calcium carbonate (the so-called biocalcification process) was discovered [2]. Based on this knowledge, in 2008, Jonkers introduced self-healing concrete with a biological agent [3]. In this novelty material, calcite-producing bacteria is in its inactive form of spores embedded together with necessary organic compounds into the concrete matrix. After a crack occurs, the spores close to the crack surfaces are activated by the penetrating moisture and organic compounds. The now active bacteria then metabolize and convert the mineral precursor compounds to calcium carbonate ($CaCO_3$), which gradually seals the crack.

Pilot studies proved the idea of self-healing biological concrete to be promising, but subsequent research highlighted several potential drawbacks. Although the applied bacteria is sporulated, experiments showed that the number of viable bacterial spores significantly decreases after approx. 7 days from casting [4]. It is generally believed that this reduction is caused by the crystalline pressures in the aging concrete. To overcome this limitation, researchers have been suggesting and investigating numerous methods of the bacteria protection: e.g. lightweight aggregates (LWA) [5–8], silica gel and polyurethane [9], standard and pH-responsive hydrogels [10, 11], melamine-based microcapsules [12], or the so called "Activated Compact Denitrifying Cores" (ACDC) particles [13].

Another problematic factor of the self-healing bio-based concrete is its potential dependence on temperature. The majority of studies was performed under optimal and stable conditions, i.e. at room temperature (around 22 °C) with a sufficient water supply. However, the average monthly temperature in the Central European region, for example, exceeds 15 °C only three times a year [14], while the average cultivation temperature for the most commonly used bacteria is up to 30 °C [15]. Thus, the presented in-vitro results may not be repeatable under more realistic outside conditions.

Only a few studies addressed this issue. Palin et al. [16] reported that cracks in cementitious composite containing a bacterial self-healing agent reduced their permeability by 95 % in the case of 0.4 mm wide cracks and by 93 % in the case of 0.6 mm wide cracks, when immersed in artificial seawater at 8 °C for 56 days. In contrast, a field study carried out by Paine et al. [17] did not report such optimistic results. In this study, a series of reinforced concrete wall panels were prepared partially with an addition of coated expanded perlite with immobilized bacteria. The panels were cracked and situated at their planned location – as a conventional retaining wall structure at highway. After 7 months, no complete crack healing could be observed, and bacterial calcite precipitation could not be precisely distinguished from the autogenous healing. As relative humidity stayed rather high throughout the experiment, researchers identified the low temperature (mostly below 15 °C) as one of the possible causes.

In the study presented in this paper, both of the aforementioned issues – the protection of bacterial spores in concrete matrix and application of the material under non-ideal conditions – are addressed. As the protective agents, two types of polymers are applied – superabsorbent polymer (SAP) powder and polyvinyl alcohol (PVA) in the form of a water solution.

The SAP powder (a material characterized by a striking absorption capacity up to hundred times its own weight) has already been investigated as an admixture for concrete, inter alia, to enhance the natural self-healing mechanisms [18, 19]. In our case, the function of SAP is twofold. It is believed that the swollen SAP particles (or voids after their drainage) could ensure enough space for bacteria to survive, and, at the same time, serve as a reservoir for the moisture needed for the bacteria metabolic activity. SAP has already been successfully applied to a similar purpose by Kua et al. [20], where the combination of SAP, biochar and bacteria led to closure of cracks up to 800 μm and higher recovery of mechanical properties compared to non-bacterial samples.

There is no mention in the existing literature of the application of PVA water solution in self-healing bio-based concrete. Although several studies have already evaluated its effect on mechanical properties, reduced water absorption or increased acid resistance of cementitious composites [21–24]. In this study, it is expected that the polymer could form a protective layer around the spores, while the increased porosity (according to previous research) would provide additional protective space for the bacterial spores.

1.2 Object of the study

The paper deals with the application and comparison of two polymer-based protective agents (SAP powder and PVA water solution) in cementitious composite containing bacteria and nutrient organic compounds. The influence of the applied protective agents on the material's properties (specifically on consistency and strengths) was determined via cement-flow table test, three-point bending, and compression test. Further, the self-healing was observed on cracked specimens, which were exposed for 28 days to various conditions (room temperature, low temperature, and freeze cycles). The healing efficiency was determined based on visual investigations using high-resolution photography, 3D scanning microscopy, and dynamic modulus recovery.

1.3 Research motivation and need for research

The presented study expands the existing research by an application of two protective agents (SAP and PVA) into the bio-based self-healing concrete. Further, the main motivation of the study was to provide a unique comparison of the material's crack-sealing potential in non-optimal in-vitro conditions. As the literature review has shown, only a limited number of studies has focused on the bio-based self-healing applicability at low temperatures, and no records of its behavior after exposition to temperatures below zero were found. Thus, this paper provides a valuable insight into the proposed material's applicability, as well as it indicates the issues on which the future research should focus on.

1.4 Aims and tasks of the study

The aims and tasks of the presented experimental study were:

- To determine the material characteristics of the proposed cement composite containing SAP and PVA protective agents.
- To evaluate the impacts of the applied protective agents on the material's crack-sealing potential.
- To describe and compare the crack-sealing potential under various conditions (room vs. low temperature).
- To determine the impact of freeze cycles on the crack-sealing, thus, to determine the survival and subsequent viability of the embedded bacterial spores.

2. Materials and methods

2.1. Materials

Ordinary Portland cement CEM I 42.5 R conforming to the standard EN 197-1 obtained from Závod Mokrá, Czech Republic was used for all of the series in the study. Fine aggregates with maximum grain size 1.0 mm and medium aggregates with maximum grain size 2.0 mm, both obtained from Provodínské písky a.s., Czech Republic, were applied.

For the mix preparation, distilled water was used in all cases for the sake of improved reproducibility of the experiment. Especially in the case of the SAP addition, the ionic composition of the applied mixing water may affect the results significantly. As demonstrated in our preliminary research [25], the absorption capacity of the applied SAP can reach up to 1.6 times higher values in distilled water than in tap water available in the laboratory location (Dejvice, Prague, Czech Republic).

2.2. The bacterial healing agent

Alkaliphilic and alkalitolerant, aerobic endospore-forming bacteria *Bacillus pseudofirmus* was used in this study. The bacterium (LMG 17944) was obtained from Belgian Coordinated Collections of Microorganisms (BCCM). The culture and sporulation media were prepared according to the BCCM prescriptions.

The calcium carbonate precipitation, thus the targeted crack-sealing, is achieved in the case of *Bacillus pseudofirmus* through degradation of organic compounds according to the following reaction [4]:

$$\text{Organic compounds} + O_2 \rightarrow CaCO_3 + CO_2 + H_2O. \quad (1)$$

It follows that it is crucial to provide the organic compounds (calcium source and metabolic activator) externally, as they are not present in the concrete mix itself. In this study, the nutrients necessary for the calcite production by the bacteria were selected based on the existing literature and own preliminary testing [26]. Yeast extract obtained from Carl Roth GmbH + Co. KG, Germany was used as a metabolic activator. Calcium lactate, $C_6H_{10}CaO_6.5H_2O$, purity ε 98 %, obtained from Carl Roth GmbH + Co. KG, Germany, was applied as a calcium source.

2.3. The protective agents

Powdered dry SAP (a cross-linked acrylamide/acrylic acid copolymer, potassium salt obtained from Evonik Industries AG, Germany) and 16 % PVA aqueous solution (consisting of 13 % polyvinyl alcohol and 4 % polyvinyl acetate, obtained from FISCHEMA s.r.o., Czech Republic) were applied as protective admixtures.

2.4. Mix design, mixing, specimens, and curing

In Table 1, mix compositions of the prepared series are shown. All of the series (including control) contained the same amount of Portland cement, fine and medium aggregates, and nutrients necessary for the biocalcification. Based on the existing literature [4, 27, 28] and own experiments [26], calcium lactate in a dosage of 3.0 % of cement weight was chosen as the calcium source. Yeast extract was applied in a dosage of 0.45 % of cement weight. Although some suggested a maximal value of 0.85 % [12], our preliminary investigations indicated an unacceptable drastic drop in the compressive strength [26].

Generally, the base value of the water/cement ratio (w/c) was 0.5. In the case of SAP series, the w/c was slightly increased (additional 15 g of distilled water per 1 g of SAP based on our previous research [25]) in order to compensate for the liquid uptake by SAP, while preserving the paste flowability. On the contrary, the amount of mixing water in the PVA series was reduced by the amount of water present in the 16 % PVA water solution. Thus, the overall w/c ratio would correspond to the w/c = 0.5.

SAP was applied at a dose of 0.5 % cement weight, 16 % aqueous PVA solution at a dose corresponding to 1 % PVA cement weight. The bacteria were applied in the form of spores (their preparation described elsewhere [30]), which were thoroughly dispersed in the corresponding amount of mixing water (BAC and BAC_SAP) or the PVA aqueous solution (BAC_PVA). The final concentration of colony-forming units (CFU) per 1 ml of the mixing water (including the amount in the PVA solution) was around 8×10^6.

Table 1. Composition of the mixtures (values in [kg/m^3].

Compound	CTRL [kg/m^3]	CTRL_SAP [kg/m^3]	CTRL_PVA [kg/m^3]	BAC [kg/m^3]	BAC_SAP [kg/m^3]	BAC_PVA [kg/m^3]
Portland cem.	586	586	586	586	586	586
Distilled water	293	337	262	293	337	262
Medium agg.	440	440	440	440	440	440
Fine agg.	1319	1319	1319	1319	1319	1319
SAP	no	2.93	no	no	2.93	no
16 % PVA	no	no	36,63	no	no	36.63
Calcium lactate	17.58	17.58	17.58	17.58	17.58	17.58
Yeast extract	2.64	2.64	2.64	2.64	2.64	2.64
Bacteria [CFU/ml]	no	no	no	8×10^6	8×10^6	8×10^6

The mixing procedure was kept identical in all cases. Yeast extract was homogenized with cement prior to mixing and calcium lactate was dissolved in mixing water. In the case of SAP and PVA series, both of the polymers were applied alongside cement and mixed prior to the aggregate and water addition.

From the prepared mixes, two types of specimens were prepared – specimens for mechanical testing and specimens for crack-sealing investigations. All of the specimens were prepared in triplicates for each mix design and testing method. Both of the types were casted in 40×40×160 mm^3 steel molds, thoroughly vibrated using a vibrating table. In the case of the specimens intended for the crack-sealing, around 20 profiled steel wires were placed in the middle of the span, approx. 1 cm from the mold top (Fig. 1) in order to ensure the stability of the crack widths.

The molds were then left at room temperature covered with plastic foil for 24 hours to harden. Thereafter, all of the specimens were unmolded and placed in a climate chamber with temperature 24 °C and relative humidity up to 95 % for 28 days. After the end of the curing period, dimensions of the specimens were thoroughly measured, and the samples were weighed.

Figure 1. Preparation of the samples with steel wires for the controlled crack creation.

2.5. Controlled cracking

In order to estimate the healing capacity of the proposed combinations of bacteria and protective methods, the prepared reinforced samples were cracked after the end of the curing period. The cracks were introduced through three-point bending using a calibrated electric loading machine. The loading rate was controlled manually and operatively altered to avoid complete destruction of the sample. The procedure layout is displayed in Fig. 2.

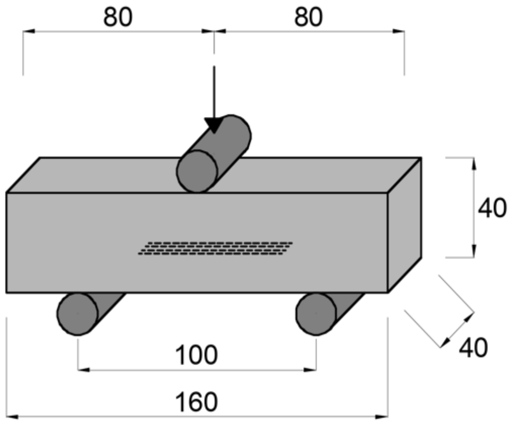

Figure 2. A layout of the crack creation through 3-point bending test.

2.6. Methodology

As already mentioned in the Introduction, the impact of the variety of conditions which may occur in real outside environment is generally neglected in the studies dealing with the bio-based self-healing concrete. To address the issue in this paper, the samples were subjected to three different conditions: ideal, low temperature and temperatures below the freezing point.

The ideal environment (25 ± 2 °C) served as the reference. This value also more or less corresponds to the highest reachable average month temperatures in the place of our research – the Central Europe region. To inspect the healing potential in the ideal conditions, the cracked samples were placed into separate plastic containers filled with tap water and left at the given temperature for 28 days. All of the series were exhibited to the ideal conditions in order to obtain a complete overview of each material's healing capacity. Thus, the contribution of bacteria and each protective method to the healing process could be determined.

The temperature of 10 °C was chosen for the investigation at low temperatures. In the place of our research, Czech Republic, the long-term air temperature normal (1981-2010) reaches and exceeds this value from May to August, i.e. in 5 months of the year, according to the data from the Czech Hydrometeorological Institute. In the case of sufficient crack sealing at this temperature, the self-healing could potentially take place for a large part of the year, thus the material could be declared applicable in the Central European region. In order to inspect this hypothesis, identically as in the ideal conditions, the cracked samples were submerged in water in plastic containers. The containers were then placed in a climate chamber with a controlled temperature of 10 °C for 28 days. In this case, only specimens with bacteria and protective polymers (i.e. BAC_SAP and BAC_PVA) and control (CTRL) were used.

An investigation of the impact of temperatures below the freezing point on the self-healing ability is a unique extension of the current state of the art. Although the crack sealing due to the metabolic activity of bacteria at freezing temperatures is not expected, it is crucial to answer the question, whether the bacteria immobilized in the cementitious material/protective polymer can withstand these conditions and restore its activity once the temperature raises. To simulate the freezing conditions, the cracked samples, prior to any water submersion, were placed into a freeze-thaw chamber. Through air flow, the temperature was precisely and gradually varied from 0 °C to –5 °C. The time of one cycle was 24 hours (Fig. 3). The samples were left at the chamber for 14 days (i.e. 14 cycles).

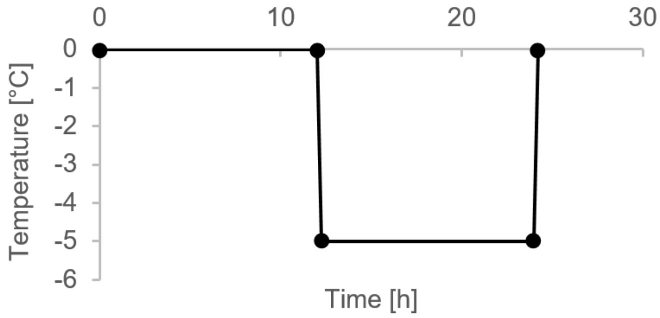

Figure 3. The temperature course of one freezing cycle.

Although such conditions do not necessarily correspond completely to reality, they are sufficiently testing a range of frequently occurring values in a relatively short test time. After the below-zero temperature cycles in the chamber, the samples were taken out and placed into water-filled containers in ideal conditions (as described above) for 28 days. As in the previous case, only specimens with protective polymers (i.e. BAC_SAP and BAC_PVA) and control (CTRL) were used.

2.7. Test methods

The main goal of this paper is to identify the properties of the proposed bacteria-polymer cementitious composites and determine their crack-sealing ability under various conditions. To obtain the information about the prepared materials, a series of mechanical and rheological tests were performed. The crack sealing potential was inspected through visual investigations and dynamic modulus recovery.

2.7.1. Consistency and mechanical tests

In order to determine the consistency of the prepared fresh cementitious composites, prior to the specimen casting, part of each paste was taken and submitted to a cement flow table test (i.e. determination of the spreading diameter). The procedure was performed according to the relevant standard [29]. Consistency was evaluated for the non-bacterial series as bacteria itself, based on the literature, was not expected to influence the property to a noticeable extent.

After the end of the curing period (i.e. 28 days from casting), the unreinforced prismatic specimens ($40 \times 40 \times 160$ mm^3) were submitted to a three-point bending test. Subsequently, a compression test was run on the halves left after the bending. In both cases, the specimens loading was run in a deflection-controlled mode using a calibrated electric loading machine. Deflection and loading force values were recorded and analyzed using software (SMAPS). The procedures were performed according to the relevant standard [32]. As well as in the case of consistency measurements, the mechanical properties were determined only on the non-bacterial series.

2.7.2. Visual inspections of the crack healing efficiency

One of the key tasks of this paper is to determine the crack sealing efficiency of the proposed cementitious bacteria-polymer composites in various conditions. In this study, the maximum sealed crack width was selected as the basic indicator of the self-healing potential. Through this value, the extent of the crack sealing can be easily compared through the individual series without the need for uniformed damages. The average maximum healed crack width ($\Delta w_{\max}$) was determined as

$$\Delta w_{\max} = \frac{\sum w_{\max}}{n}, \qquad (2)$$

where $w_{\max}$ is the maximum crack width that was sealed in each specimen and n is the number of specimens in each series.

In order to document the development of the crack sealing, all of the cracked reinforced specimens were subjected to high-resolution photography at the beginning of the healing period, and after 28 days in the respective environment. To obtain further information about the crack-closure, selected specimens were also additionally scanned with a 3D scanning optical microscope.

2.7.3. Dynamic Young's modulus recovery

The crack-sealing in the bio-based concrete primarily aims to the extension of the structure's durability, thus improvement of the material's water-tightness through reduction of the crack area. However, recovery of mechanical properties would surely be a welcome side effect. Furthermore, the information about the changed properties may appropriately supplement the information obtained from the visual assessments.

In this paper, the dynamic modulus was measured on all of the reinforced specimens before the cracking, after the cracking and after the healing period. For the quantity evaluation, the Resonance Frequency dynamic methodology was applied.

The Resonance Frequency dynamic method is a non-destructive test for determination of dynamic modulus (E_d) based on the responses obtained from a vibrating signal induced in the specimen. The resonance frequency of the specimen, which produces the maximum amplitude of vibration, is then used to calculate the corresponding E_d value [31]. For the evaluation, the Brüel & Kjaer assembly (measurement station type 3560-B-120, type 4519-003 acceleration transducers, an 8206 impact hammer type, and a computer) was used as described elsewhere [32].

The dynamic Young's modulus was evaluated based on the longitudinal natural frequency of the samples as

$$E_{d,l} = \frac{4lmf_l^2}{bt}, \quad (3)$$

where $E_{d,l}$ is the dynamic Young's modulus [Pa], l is the sample length [m], m is the sample mass [kg], f_l is the basic longitudinal natural frequency of the sample [Hz], b is the sample thickness [m], and t is the sample height [m] [32].

3. Results

3.1. Consistency

The comparison (Tab. 2) between the spreading of the control mix (CTRL) and mix containing SAP (CTRL_SAP) shows that the applied amount of extra mixing water (15 g distilled water per 1 g SAP) leads to a paste with consistency almost identical to the control one. Thus, the SAP liquid uptake in this specific mix design is close to the "extra mixing water" value which was applied. However, it must not be forgotten that due to the extreme sensitivity of the SAP absorption to the ionic composition of the soaking solution, the results might vary greatly with different w/c ratio or nutritive additions (calcium lactate and yeast extract), see our previous study [25].

The addition of PVA water solution led to appreciably more flowable paste compared to the control (Tab. 2). This result contradicts with the majority of studies where the PVA addition generally caused increased viscosity but reduced consistency [23]. However, the studies dealing with the water-soluble cementitious composites frequently use much lower w/c ratios (close to 0.3) [22, 33]. Thus, the results cannot be compared directly as the overall water content might influence the PVA behavior in the material greatly.

Table 2. The results of the flowability test – the initial spreading diameter and final spreading diameter after the dropping.

Mix type	Initial spreading [mm]	Final spreading [mm]
CTRL	80	160
CTRL_SAP	80	159
CTRL_PVA	125	183

3.2. Tensile and compressive strength

The mechanical tests revealed several important aspects of the cementitious composite with polymer additions applied in this study. The mean values of the measured quantities are shown in Fig. 3. Firstly, the proposed dosage of the nutritive compounds (3 % wt. of cement of calcium lactate and 0.45 % wt. of cement of yeast extract) proved to be suitable as the compressive and tensile strength reached sufficiently high values (mean values 39.4 MPa and 6.4 MPa, respectively).

The series CTRL_SAP evinced satisfactory behavior in tension. Its tensile strength reached slightly higher values compared to the control mix (the mean value about 7 % greater). On the other hand, the applied alterations of the mix caused rather significant drop in the compressive strength. The mean value of the compressive strength was about 30 % lower compared to the control.

The addition of 1 % wt. of cement of PVA (in the form of 16 % water solution) in the series CTRL_PVA resulted in a drastically weaker material in both cases. The tensile strength reached only 44 % of the control mix strength, the compressive strength as low as 21 %. This finding contradicts with the results presented elsewhere as generally, the compressive strength decreased similarly as in our case, but the tensile strengths tended to be improved [21, 32].

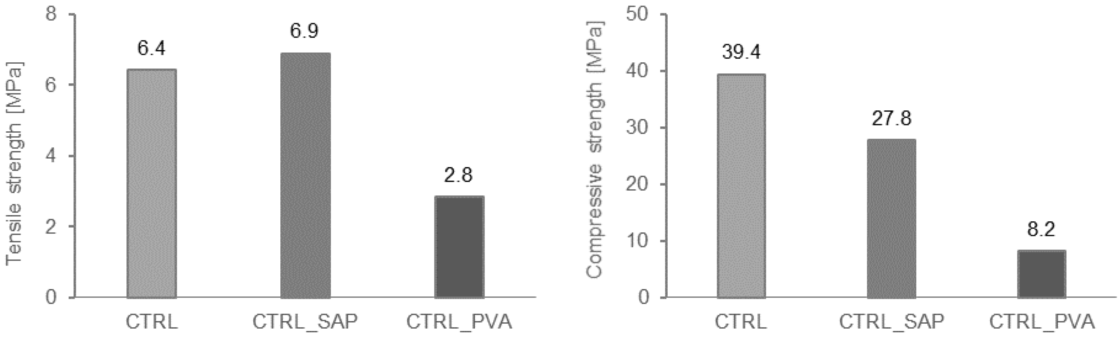

Figure 4. The mean values of tensile (left) and compressive (right) strengths of the non-bacterial mixes obtained through the mechanical tests.

3.3. Visual inspections of the crack healing efficiency

In this work, we sought to establish the applicability of the proposed bio-based self-healing concrete in other than ideal in-vitro conditions, thus extending the scope of the majority of earlier studies. In Fig. 5, for the sake of completeness, all values of the average maximum sealed crack width ($\Delta w_{\max}$) that could be identified in each series are summarized.

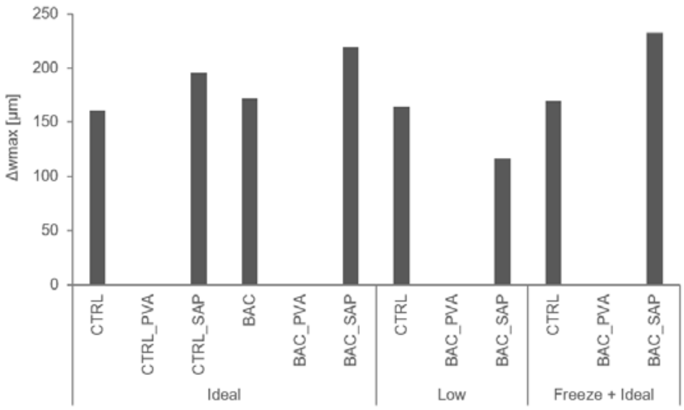

Figure 5. An overview of the average maximum healed crack widths ($\Delta w_{\max}$) in each series.

3.3.1. Healing at ideal temperature

The widest range of the cement composite mix designs was subjected to the healing in ideal conditions (i.e. room temperatures as described in the Methodology section). In general, the data suggest that detectable crack-sealing took place in all of the prepared series except the ones containing liquid PVA (Fig. 5). Further, in Fig. 6 and Fig. 7, a selection of the high-resolution photography results is provided.

In the reference series (CTRL), the value of $\Delta w_{\max}$ reached 161 μm. As in the CTRL series no enhancement of the self-healing capacity was applied, this value can be considered achievable through the natural autogenous crack-sealing ability of the cementitious material in this study.

A slightly higher value (172 μm) was recorded when bacterial spores without any protection (BAC) were incorporated into the cementitious composite. This would indicate that in this study, the natural autogenous crack-sealing potential could be increased by the bacteria-driven $CaCO_3$ precipitation by around 7 %.

In the ideal conditions, the widest crack parts were sealed in the case of the SAP addition. In the composite with SAP alone (CTRL_SAP), the $\Delta w_{\max}$ increased to 195 μm. When a combination of SAP and bacterial spores was applied (BAC_SAP), the $\Delta w_{\max}$ reached as high as 219 μm. These results would indicate the overall positive impact of the SAP addition to the self-healing mechanisms as mentioned in the Introduction.

Furthermore, the difference between the series with only SAP and SAP-bacteria combination was higher (around 12 %) compared to the difference between the reference series (CTRL) and series containing the unprotected bacteria (BAC). Thus, these results may suggest the possible SAP protective potential as it seems to improve the biocalcification process itself.

In this study, as mentioned previously, the self-healing potential of PVA-based cement composite series (CTRL_PVA and BAC_PVA) showed to be completely disappointing as no crack-sealing was detectable in the case of the liquid PVA addition. This result is somewhat surprising, as the PVA presence seems to not only inhibit the biocalcification, but also the natural autogenous self-healing.

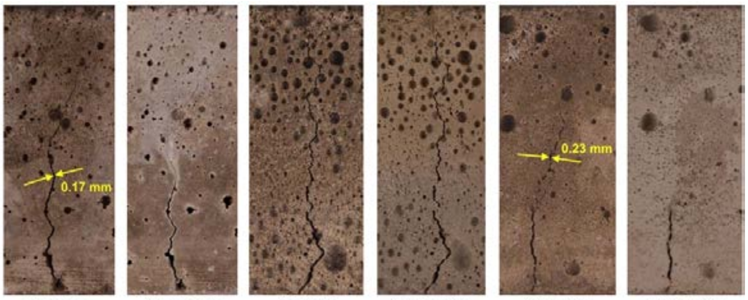

Figure 6. High-resolution photography before (0 days) and after the healing period (28 days) in ideal conditions of the non-bacterial samples. The maximum healed crack width on the individual samples is marked.

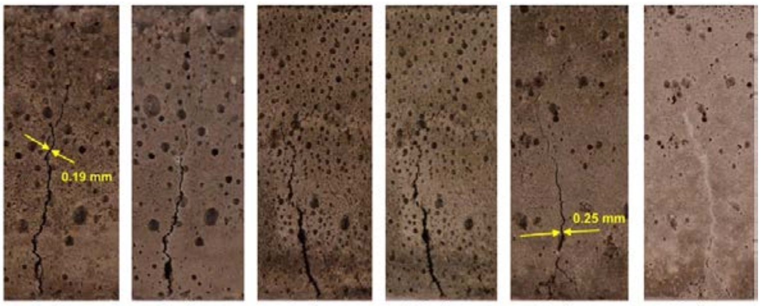

Figure 7. High-resolution photography before (0 days) and after the healing period (28 days) in ideal conditions of the bacterial samples. The maximum healed crack width on the individual samples is marked.

In Fig. 8, details of selected cracked specimens after the healing period are presented. The images are in line with the previous findings. In the case of BAC and BAC_SAP, the cracks are almost completely sealed with white crystalline precipitates. In the cracked BAC_PVA specimen, some formation of the precipitates can be also seen on the crack surfaces; however, closing of the crack was not achieved.

Figure 8. Images obtained using a 3D scanning microscope.

3.3.2. Healing at low temperature

As outlined in the Introduction, the problematic functionality of the bio-based self-healing concrete at lower temperatures was frequently mentioned in earlier studies. In our case, the findings are in line with the pessimistic presumptions (see Fig. 5 for complete overview and Fig. 9 for selected cracks).

In the 10 °C environment, the autogenous crack-sealing detected in the case of CTRL did not noticeably differ from the values achieved in the ideal conditions (Δw_{max} = 165 μm). Interestingly, in the

BAC_SAP series, the $\Delta w_{\max}$ dropped to 117 µm. Thus, it seems that not only the bacteria-driven biocalcification was limited at low temperatures as expected, but also the results indicate that the positive impact of SAP to the self-healing may be inhibited by the temperature as well. Further, it seems that the SAP at low temperatures possibly even limits the natural autogenous crack-sealing capacity as the $\Delta w_{\max}$ was even lower by 30 % compared to the control series.

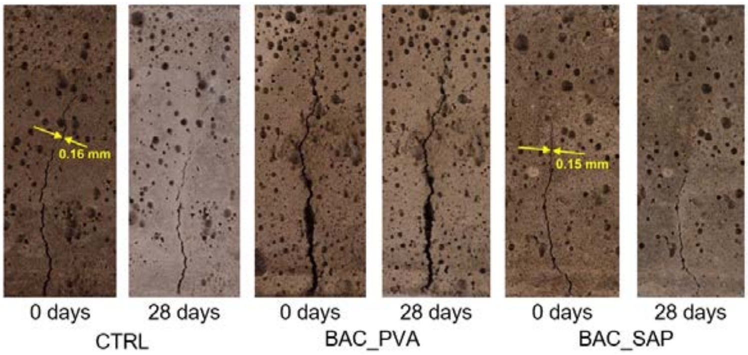

Figure 9. High-resolution photography before (0 days) and after the healing period (28 days) at low temperature of the bacterial (BAC_PVA and BAC_SAP) and non-bacterial (CTRL) samples. The maximum healed crack width on the individual samples is marked.

3.3.3. Healing at ideal temperature after exposure to freeze cycles

In the place of our research (Central European region), sub-zero temperatures are common in the winter season. Thus, as outlined in the Introduction, the regain of metabolic activity of bacteria incorporated in the cementitious matrix is a crucial factor affecting the bio-based self-healing concrete applicability in our climate zone.

In this study, the experiment involving freeze cycling followed by a healing period at room temperature (see Methodology section) yielded interesting results. In Fig. 5, a complete overview of the $\Delta w_{\max}$ is shown and in Fig. 10, a selection of the high-resolution photography of the cracked specimens exhibited to the respective environment is provided.

From Fig. 5 it can be seen that, interestingly, the $\Delta w_{\max}$ reached in both CTRL and BAC_SAP even slightly higher values compared to the series without the freeze treatment (170 and 233 µm, respectively). However, the difference between the two mentioned series remained almost identical in both of the environments i.e. around 35 % increase in the case of BAC_SAP. Thus, the bacteria viability was not negatively affected by the freezing cycles, possibly thanks to the SAP that served as a sufficient protective method.

Consistently with the previous results, even after the freeze treatment, no crack-sealing could be observed in the series containing liquid PVA as illustrated in Fig. 5 and Fig. 10.

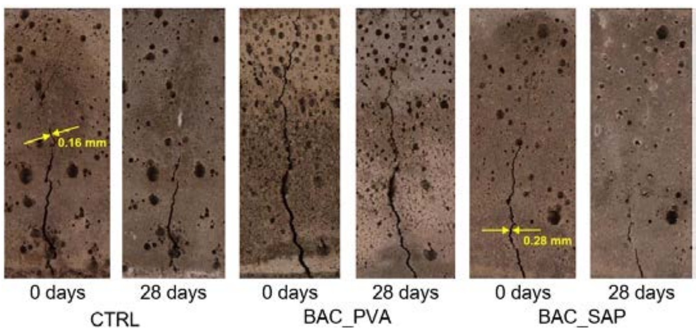

Figure 10. High-resolution photography before (0 days) and after the freeze cycles and healing period (28 days) in ideal conditions of the bacterial (BAC_PVA and BAC_SAP) and non-bacterial (CTRL) samples. The maximum healed crack width on the individual samples is marked.

3.4. Dynamic Young's modulus recovery

In order to detect possible recovery of mechanical properties (namely dynamic modulus of elasticity E_d) caused by the crack-sealing, the non-destructive resonance frequency dynamic method was applied on the reinforced specimens in this study. In Fig. 11, the mean values of $E_{d,l}$ evaluated from longitudinal vibration measured on the specimens before cracking can be seen. These values more or less correspond to the tendencies noticeable from the mechanical tests – the addition of PVA generally caused drop of the monitored quantity, whereas the SAP series values were around the control values. After the controlled cracking, the value of $E_{d,l}$ in all of the series was zero as expected.

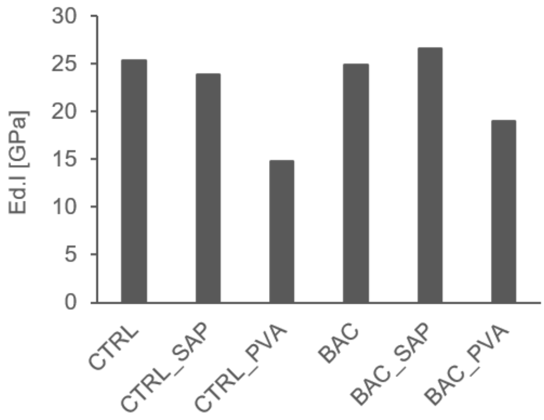

Figure 11. The mean values of the dynamic modulus of elasticity E_d measured on the uncracked specimens.

Measurements after the end of the healing period were far from complete as it was possible to detect the longitudinal frequency only for a fraction of the samples; for the rest $E_{d,l}$ remained zero. Overview of all the successfully measured samples is given in Table 2. Provided that the measurement of the longitudinal frequency could be accomplished only if the filling of the crack was sufficiently rigid and solid, the data would suggest that the combination of bacteria and SAP leads to the most reliable crack-sealing as the majority of measurable samples was from the BAC_SAP series in all of the temperature conditions. Further, the recovery rate (healed/uncracked specimen) seemed to be consistently the highest in the case of BAC_SAP series (as much as 51 %).

Table 2. Values of E_d measured on uncracked specimens, after cracking, and after the healing period. Recovery represents the ratio Healed to Uncracked values.

Environment	Specimen n.	E_d [GPa]			Recovery [%]
		Uncracked	Cracked	Healed	
Ideal	CTRL 1.3	26	0	9	35
	CTRL_SAP 1.1	23	0	5	23
	BAC 1.1	25	0	8	32
	BAC 1.2	25	0	10	38
	BAC 1.3	24	0	6	25
	BAC_PVA 1.1	19	0	6	33
	BAC_SAP 1.1	27	0	10	39
	BAC_SAP 1.2	27	0	10	38
	BAC_SAP 1.3	26	0	13	51
Freeze	CTRL 3.1	27	0	8	31
	BAC_SAP 3.1	27	0	9	34
	BAC_SAP 3.2	27	0	6	22
Low	BAC_SAP 2.2	26	0	7	27

4. Discussion

In this paper, two different compositions of the bio-based self-healing concrete were proposed and investigated. Firstly, the materials characteristics of the proposed cementitious composites were determined. The flowability table test showed that when enriching the bio-based cement composite with SAP, a dose of extra mixing water (15 g distilled water per 1 g SAP) ensures preservation of the paste workability. The test further revealed that addition of PVA (1 % wt. of cement) leads to a more flowable paste compared to the control.

The performed mechanical tests provided information about the strengths of the proposed cement composites. According to the 3-point bending test, the addition of 0.5 % wt. of cement of SAP and extra mixing water lead to improvement of the material's tensile strength. On the other hand, the addition of liquid PVA caused a significant drop of the tensile strength. The compressive test showed that the cement composite containing SAP in the applied dosage is a slightly weaker material in compression compared to the control. In the case of PVA application, similarly to the tensile strength, the compressive strength was lowered dramatically compared to the reference in our study.

The main aim of this paper was to provide comparison of the crack-sealing potential of the bio-based self-healing concrete containing SAP/PVA in other than ideal conditions. First of all, however, it should be noted that the overall efficiency of the crack closure in this experiment is generally lower compared to the values reported elsewhere. In our case, the maximum healed cracked width was around 300 μm (BAC_SAP), whereas other studies described sealing of cracks with widths up to 400-700 μm [5, 8, 20]. However, we must take into consideration that even scattering of the autogenous crack-sealing itself throughout the studies is considerable. This phenomenon shows how complex it is to quantify the material's efficiency, as it is influenced by a large number of, in most cases, volatile factors (such as mix design, w/c ratio, state of the applied bacteria, possibly the cement chemistry etc.).

At the ideal temperature, the visual investigations indicated that SAP may increase the natural autogenous crack-sealing capacity as the observed value $\Delta w_{\max}$ was around 20 % higher than control. This finding is in line with the results presented elsewhere [18, 19]. Further the SAP presence seemed to improve the biocalcification efficiency based on the visual inspections – the $\Delta w_{\max}$ of bacteria-SAP composite reached around 35 % higher value compared to control. This conclusion was also presented in an earlier study [20]. The investigation of dynamic modulus recovery indicated similar tendencies at the ideal temperature. The majority of the cracked specimens in which the value $E_{d,l}$ could be measured belonged to the BAC_SAP series.

Further research should be focused on the bacteria-SAP combination in conditions with a lower/inconsistent water supply. In this environment, the positive impact of SAP on autogenous crack-sealing and biocalcification might be even more pronounced as the SAP absorption capacity could ensure the needed moisture for both of the self-healing mechanisms.

Interestingly, in our study, the SAP had a slight negative impact on the self-healing at 10 °C based on the visual investigation. Although it is possible to assume that the biocalcification process was completely inhibited at this temperature, the SAP sample showed even worse results than the control. However, this finding completely contradicts with the dynamic modulus measurements as $E_{d,l}$ could be recorded only on the specimen containing bacteria and SAP.

As to the knowledge of the authors, this experiment is first of its own kind, and the mechanisms of the polymer's functionality in the cement composite at low temperatures are unknown. However, the finding provides an interesting indicator of what the future research should be focused on in terms of the SAP impact on the autogenous self-healing.

Unique results were obtained by research of the self-healing in the proposed materials after freeze cycles. In the case of SAP-bacteria composite, the visual inspections showed that the exposure to temperatures below zero did not result in any decrease in the crack-sealing capacity. After 28 days of healing in ideal conditions, the detectable crack-sealing was similar (or even higher) to the series non-treated by the freeze cycling. Similar results were indicated by the dynamic modulus recovery measurements as, again, the majority of successfully measured specimens were from the BAC_SAP series.

In our study, the impact of liquid PVA to the self-healing was disastrous in all of the healing environments. Not only it seems to inhibit the biocalcification process, but also not even any noticeable autogenous crack-sealing occurred in any of the monitored samples. The mechanism behind the negative impact is yet to be discovered as no records of its application in self-healing concrete was found in the existing literature. A possible explanation could lay in a decreased level of cement hydration caused by the polymer. This would also correspond with the results of mechanical characteristics as both the tensile and compressive strength were significantly lower compared to the control. However, further research dealing with this issue would be needed.

5. Conclusions

In the current study, the combination of bacteria *Bacillus pseudofirmus*, nutritional compounds, and SAP or PVA was applied in cement composite in order to evaluate the biologically enhanced material's self-healing potential in various healing conditions. The following conclusions can be drawn based on the current experimental investigation:

- SAP in all probability has a positive impact on the natural autogenous crack-sealing;

- In this paper, the SAP addition seemed to improve the biocalcification process, thus the bacteria driven crack-sealing;
- The SAP functionality might be limited at lower temperatures; however, more research on the exact mechanism is needed;
- The efficiency of the proposed self-healing cement composite containing the combination of SAP and bacterial healing agent did not seem to be affected by the freeze cycles;
- The application of liquid PVA turned out to be unsuitable from the point of view of both material characteristics and self-healing efficiency.

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