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Self-healing performance of biogranule containing microbial self-healing concrete under intermittent wet/dry cycles

Biyogranül içeren mikrobiyal kendini onaran betonların aralıklı ıslak/kuru döngülerde kendini onarma performansı

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Self-Healing Performance of Biogranule Containing Microbial Self-Healing Concrete Under Intermittent Wet/Dry Cycles

Highlights

- *Microbial self-healing performance of self-protected biogranule containing cementitious composite was investigated under consecutive a week long wet/dry cycles*
- *Dry periods have severe negative impact on autogenous healing performance while they have slightly positive contribution to the microbial self-healing*
- *In 4 weeks, cracks as wide as 400 μm were effectively healed only in microbial self-healing concrete*
- *Microbial self-healing enables 44% better water tightness regain when compared to autogenous healing*

Graphical Abstract

Under wet/dry cycles, mirobial self-healing performance of biogranule containing cementitious composites was revealed for the first time.

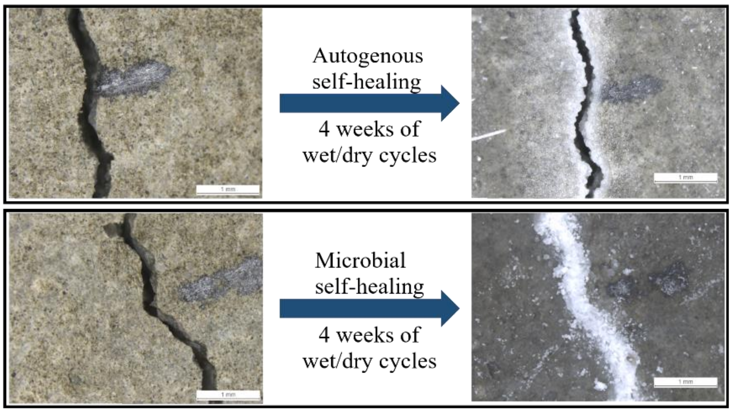

Figure. Biogranule containing concrete reveals enhanced self-healing under wet/dry cycles

Aim

Reveal self-healing performance of biogranule containing bioconcrete under wet/dry cycles.

Design & Methodology

Bacteria content was set to 1.00% w/w cement. Microbial self-healing performances were compared with the autogenous healing performances of abiotic control and plain control.

Originality

Biogranule containing concrete was investigated under wet/dry cycles for the first time.

Findings

Bioconcrete can heal cracks as wide as 400 μm with a 44% better water tightness regain.

Conclusion

Biogranule containing self-healing concrete can be used not only in completely immersed structures but also in structures where spraying or intermittent wetting occurs.

Declaration of Ethical Standards

The authors of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

Biyogranül İçeren Mikrobiyal Kendini Onaran Betonların Aralıklı Islak/Kuru Döngülerde Kendini Onarma Performansı

Araştırma Makalesi / Research Article

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ÖZ

Beton yapıların bakımına yönelik olarak yürütülen ve yoğun iş gücü isteyen izleme/onarım faaliyetlerinin minimuma indirilebilmesi için, kendini algılayan ve kendini onaran betonların geliştirilmesi önem arz etmektedir. Kendini onaran betonların bir çeşidi beton çatlakları içerisinde kalsiyum karbonat çökelmesini tetikleyen mikrobiyal ajanların kullanılması ile elde edilebilir. Son zamanlarda, nitrat indirgeyen mikroorganizmalardan oluşan biyogranüller yeni nesil mikrobiyal ajanlar olarak sunulmuş ve biyogranül içeren numuneler batık koşullarda iyi bir çatlak onarım performansı göstermiştir. Ancak, bu numunelerin çeşitli beton yapıların maruz kaldığı aralıklı ıslanma koşulları altında performansları bilinmemektedir. Bu çalışmada, biyogranül içeren mikrobiyal harç örneklerinin aralıklı ıslak/kuru koşullar altında kendini onarma performansları sunulmaktadır. Kurum içinde üretilen biyogranüller harç numunelerine çimento ağırlığınca %1.45 w/w (% 1.00 bakteri w/w çimento) dozunda eklenmiş ve çatlatılan harç numunelerindeki 50 ila 600 µm arasındaki çatlakların birbirini izleyen ıslak/kuru şartlarda kendiliğinden iyileşme performansları incelenmiştir. Islak/kuru döngülü 4 haftanın sonunda, biyoharç numunelerinde 400 µm genişliğine kadar olan çatlaklar etkili bir şekilde iyileşmiştir. Daha iyi kendini onarma performansları sayesinde biyoharç numunelerinin su sızdırmazlık geri kazanımı kontrol numunelerine göre %44 daha fazla gerçekleşmiştir. Genel olarak, biyogranüllerin püskürme veya aralıklı ıslanma koşullarına maruz kalan yapılarda uygulanmaya yönelik olarak geliştirilebilecek kendini onaran biyobetonlar için kullanışlı olduğu görülmüştür.

Anahtar Kelimeler: Kendini onaran beton, biyomineralizasyon, bakteri miktarı, ACDC granül, bakterili beton.

Self-Healing Performance of Biogranule Containing Microbial Self-Healing Concrete Under Intermittent Wet/Dry Cycles

ABSTRACT

Development of self-sensing and self-healing concrete is essential to minimize the labour-intensive monitoring and repair activities conducted for the maintenance of concrete structures. A type of self-healing concrete can be achieved by using microbial agents that induce calcium carbonate precipitation inside a concrete crack. Recently, biogranules consist of nitrate reducing microorganisms were presented as a new generation microbial healing agent and biogranule containing specimens revealed decent healing performance under completely submerged conditions. However, their performance under intermittent wetting conditions, a common case for various concrete structures, remains unknown. This study presents the self-healing performance of biogranule containing biomortar specimens under intermittent wet/dry conditions. In-house produced biogranules were incorporated into mortar specimens at a dose of 1.45% w/w cement (1.00% of bacteria w/w cement) and self-healing performance of cracked specimens were investigated under alternating wet/dry conditions for a crack width range of 50 to 600 µm. Upon alternating wet/dry treatment for 4 weeks, cracks up to a 400 µm crack width were effectively healed in biomortar specimens. Their water tightness regain was 44% better than control specimens due to their enhanced healing performance. Overall, non-axenic biogranules appear to be useful in development of self-healing bioconcrete for applications under spraying or intermittent wetting conditions.

Keywords: Self-healing concrete, biomineralization, bacteria content, ACDC granule, bacteria-based concrete.

1. INTRODUCTION

Microcracks poses a major threat against the durability of concrete structures as they facilitate external attacks to the concrete matrix and the reinforcement bar, which further increase the maintenance costs and decrease the service life of the structure. Current maintenance activities involve labour and product costs as well as indirect costs due to partial or complete interference of the regular services of the structures. Closure of tunnels, bridges, parking lots and related traffic jam can be given as examples for the latter. Current maintenance activities can also be criticised in terms of their sustainability as commercially available crack repair products are mostly

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petroleum-based chemicals such as epoxy and polyurethane fillers. Self-sensing and self-healing concrete which minimizes external maintenance activities appeared as an alternative to traditional concrete and various types of self-healing concrete were developed [1]. Incorporating superabsorbent polymers, microcapsules or glass capsules containing various types of polymers, shape memory alloys, microfibers or natural fibers and calcium carbonate precipitating bacteria can be mentioned among the commonly used approaches for the development of different types of self-healing concrete [1]. Among them microbial self-healing concrete became prominent as the healing product in this type of concrete is calcium carbonate which is a highly compatible mineral with the concrete matrix [2].

In microbial self-healing concrete, self-healing capability is obtained by exploiting microorganisms that can induce calcium carbonate precipitation. The majority of microorganisms can induce calcium carbonate precipitation under given suitable conditions [3]. The concentration of calcium ions and the presence of biodegradable organic matter are the two key external factors governing the microbial induced calcium carbonate precipitation (MICP) [4]. The pH of the environment can also be critical in MICP when certain microbial activities such as aerobic respiration or carbon dioxide hydrolysis are of concern, since MICP through these metabolisms strictly rely on external alkalinity and can only occur in highly alkaline environments [5]. Therefore, under alkaline concrete environment, even more metabolic pathways may lead to the precipitation of carbonate.

Number of studies on microbial self-healing concrete increased in recent years. Those studies successfully dealt with the key challenges in application of bacteria in cementitious composites, such as osmotic stress, thermal stress and enzyme inactivation [6–9]. Aerobic respiration, urea hydrolysis, carbon dioxide hydrolysis and biogenic nitrate reduction are the state-of-the-art metabolisms that are suggested to develop microbial self-healing concrete [5]. Corresponding microorganisms revealing promising healing results are summarized in Table 1. Owing to their significant carbonate yield and alkalinity production, ureolytic axenic cultures are the most prominent microorganisms investigated for development of microbial self-healing concrete. However, urea hydrolysis can be criticised for its ammonium production (Eq. (1)) as the produced ammonium attacks to the cementitious matrix, decreases pH and pave the way for reinforcement bar corrosion. Moreover, ammonia, the conjugate base of ammonium, is toxic for the aquatic life [10]. Considering these drawbacks, ureolytic bacteria might not be useful for concrete applications in the marine environment.

$$CO(NH_2)_2+2H_2O \xrightarrow{\text{Bacterial urease}} 2NH_4^{+}+CO_3^{2-} \tag{1}$$

MICP through either aerobic respiration (Eq. (2)) or carbon dioxide hydrolysis (Eq. (3)) metabolism completely rely on external alkalinity (Eq. (4)) as these processes can only produce carbonic acid and bicarbonate (Eq. (2) and Eq. (3)). Due to their dependence on external alkalinity these processes sacrifice concrete pH for crack healing and facilitates carbonation of concrete.

$$Ca(C_3H_5O_3)_2+6O_2 \rightarrow Ca^{2+}+4H_2CO_3+2HCO_3^{-} \tag{2}$$

$$H_2O+CO_2 \xleftrightarrow{\text{Carbonic anhydrase}} 2H_2CO_3 \leftrightarrow HCO_3^{-}+H^{+} \tag{3}$$

$$HCO_3^{-}+2H_2CO_3+3Ca(OH)_2 \rightarrow 3CaCO_3+5H_2O+OH^{-} \tag{4}$$

Among the investigated metabolic pathways, nitrate reduction appears to be the most promising for further investigations as it does not lead to production of toxic by-products and not rely on external alkalinity (Eq. (5)). On the contrary, nitrate reduction inherently produces alkalinity as well as nitrite ions (Eq. (6)), as an intermediate product, which inhibits reinforcement bar corrosion during crack healing [11]. Therefore, nitrate reduction pathway appears to be the most promising pathway for development of microbial self-healing concrete for marine applications.

$$5Ca(HCOO)_2 + 4NO_3^{-} \rightarrow 2N_2 + 6HCO_3^{-} + 4CaCO_3 + 2H_2O + Ca^{2+} \tag{5}$$

$$Ca(HCOO)_2+2NO_3^{-} \rightarrow Ca^{2+}+2NO_2^{-}+2HCO_3^{-} \tag{6}$$

In early pioneering microbial self-healing studies, the use of axenic cultures was preferred, as those studies mainly concern about the proof of concept by investigating various metabolic pathways. The costs of the reported type of microbial self-healing concretes reach up to 2400 EUR/m^3 [12]. Such high prices are mainly due to the aseptic production processes of axenic cultures and essential protective carriers (i.e. microcapsules, porous carriers, etc.) for incorporation of the microorganisms into concrete [12]. As a cost-efficient alternative to axenic cultures, non-axenic biogranules, so called Activated Compact Denitrifying Core (ACDC) were proposed as a new generation microbial healing agent [2]. Apart from their 10 to 20 times lower production cost [2], these microbial granules are composed of self-immobilized bacteria and mineral layers, thus they do not require any additional protective carrier for concrete incorporation [13]. These biogranules are incorporated into concrete as a dry powder which makes them more practical for applications in situ when compared to bacterial solutions. In their dry form, biogranules also have a longer shelf-life compared to spore containing solutions used in earlier studies.

ACDC biogranules, that are particularly granulated for concrete implementation, were determined to be compatible with cement matrix and provide crack healing performance at bacteria dose of 0.50% w/w cement [2,14,15]. Additionally, in marine conditions, ACDC biogranules simultaneously inhibit reinforcement bar corrosion and heal 300-μm-crack in 28 days [11].

In marine environment, members of concrete structures are exposed to different wetting conditions such as complete submersion, spraying or wet/dry cycles. In earlier studies, microbial self-healing was investigated

Table 1. Proposed microbial healing agents after their successful application for development of microbial self-healing concrete

Study	**Bacterial strain - pathway**	**Protective carrier**
Wiktor and Jonkers [16]	*Bacillus alkalinitrilicus* – aerobic oxidation of organic carbon	Light weight aggregates
Wang et al. [9,17]	*Bacillus sphaericus* – urea hydrolysis	Hydrogels Microcapsules
Silva et al. [7]	Cyclic EnRiched Ureolytic Powder (CERUP) – urea hydrolysis	Self-protected culture
Ersan et al. [2,18]	*Diaphorobacter nitroreducens* – anoxic oxidation of organic carbon (nitrate reduction metabolism)	Granular activated carbon
	Activated compact denitrifying core (ACDC) – anoxic oxidation of organic carbon (nitrate reduction metabolism)	Self-protected granular culture
Palin et al. [6]	*Bacillus halmapalus* – aerobic oxidation of organic carbon	Calcium alginate beads
Tziviloglou et al. [19]	*Bacillus cohnii* – aerobic oxidation of organic carbon	Expanded clay
Khaliq and Ehsan [20]	*Bacillus subtilis* – aerobic oxidation of organic carbon	Light weight aggregates
Alazhari et al. [21]	*Bacillus pseudofirmus* – aerobic oxidation of organic carbon	Expanded perlite

mostly for fresh water immersion. In couple of studies, crack healing performance of microbial self-healing concrete was also investigated under wet/dry treatment cycles [9,19,22]. Wang et al. [9,22] investigated wet/dry cycles by applying 1 hour wet and 11 hours dry periods. Tziviloglou et al. [19] investigated wet/dry cycles by applying 12 hours wet and 12 hours dry periods. In those studies, the dry cycle durations were less than a day (i.e. 11 or 12 hours) which might not be enough to observe the exact effect of dry periods on bacterial activity and crack healing. Wang et al. [9,22] even coupled their bacteria together with superabsorbent hydrogels which might interfere with the clear understanding of the effects of the dry periods in such short dry periods due to the slow release of the absorbed water from hydrogels. Therefore, in order to identify the actual effect of dry periods on crack healing performance of bio-based concrete, longer durations between cycle switches are essential. In addition to that, as previous studies were conducted by using only axenic cultures, the crack healing performance of biogranule containing cementitious composites under wet/dry cycles still remains unknown. Regarding to these unknowns, in this study, we present the crack healing performance of biogranule containing specimens under alternating a week-long wet/dry cycles.

2. MATERIALS AND METHOD

2.1. Self-Protected Biogranules as Microbial Healing Agents

Biogranules with an activated denitrifying core, namely ACDC, were used as the microbial healing agent throughout the tests. Biogranules were cultivated in a cylindrical sequencing batch reactor (effective h= 60 cm, Ø= 12.4 cm and 50% volume exchange ratio). Old dry biogranules, which had been cultivated in previous studies [2,14] were ground to a size of less than 0.212 mm (minimum particle size for a cluster of bacteria to be considered as a biogranule) and used to seed the new reactor, because the core bacteria of the old granules were the main seed for cultivation of the fresh biogranules used in this study and by destroying the granular structure of the seed, resuscitation period of the core bacteria could be shortened. The bioreactor was operated with four identical batch-cycles/day. Each cycle consisted of three periods namely, anoxic period (180 minutes), aerobic period (175-180 minutes) and settling period (0-5 minutes). Anoxic period was obtained by 120 minutes simultaneous fill/draw period with an upflow influent flow rate of 0.5 mL/s and an extra 60 minutes of sole anoxic period. The aerobic period was obtained by aeration with an upflow air velocity of 0.8 cm/s. The duration of the settling period was decreased gradually from 5 minutes to less than a minute in order to wash-out small flocs and non-granule forming microorganisms.

During the anoxic period, the reactor was fed with an alkaline minimal nutrient medium to enable cultivation of resilient microorganisms that can survive the micronutrient deficient and alkaline concrete environment. The minimal nutrient solution was composed of 47.63 mM NaHCOO, 3.90 mM $NaNO_3$, 1.63 mM $Ca(NO_3)_2.4H_2O$, 0.36 mM $MgSO_4.7H_2O$ and 0.09 mM KH_2PO_4. The pH of the feed solution was set to 10.2 by using 10 M NaOH solution.

After 60 days of operation, the first batch of biogranules were harvested and dried in a ventilated electric oven at 60ºC for 48 hours. These granules were fresh new ACDC granules cultivated from old dry ACDC granules and revealed similar properties [13]. Upon drying, granules were further allocated into three portions based on their particle sizes (0.45 to 2 mm) and stored in closed containers at room temperature. The allocated portions had sizes of 0.45 to 0.85 mm, 0.85 to 1.00 mm and 1.00 to 2.00 mm. Dry granules having particle sizes either more than 2 mm or less than 0.45 mm were returned to the bioreactor.

Representative samples were taken from the dry granules and tested for volatile solid content. Volatile solid content of the dry biogranules was used to determine the bacteria content in a harvested batch. VS analyses were conducted according to the standard methods [23]. Note that 70% of the biogranule content was bacteria and the rest was inorganic matter.

2.2. Chemical Admixtures for Microbial Activity and Crack Healing Process in Mortar

Produced ACDC granules require carbon as electron donor and nitrate as electron acceptor for their growth and energy metabolism (Eq. (5)). Growth, spore germination in particular, and energy production are essential microbial activities for microbial self-healing of cracks in concrete. Therefore, commercially available concrete admixtures, namely, calcium formate (CF) and calcium nitrate (CN), were added into the biomortar specimens as sources of electron donor and electron acceptor, respectively. All the experiments were conducted by using identical amount of CF and CN to clearly distinguish the compatibility of biogranules with the mortar matrix. CF and CN doses were decided based on a previous study which evaluated the bioavailability of nutrients in aquatic environment at various nutrient incorporation doses [24].

2.3. Assessment of the Self-Healing Performance of Different Mortars Under Wet/Dry Cycles

Plain control specimens, abiotic control specimens and self-healing biomortar specimens were prepared and tested. Plain mortar specimens were used as reference. Abiotic control specimens were used to distinguish the effect of chemical admixtures. Biomortar specimens were used to determine the influence of biogranule content.

2.3.1. Preparation of cracked mortar specimens and their treatment conditions

Series of plain control, abiotic control and biomortar specimens (30 × 30 × 340 mm) with an embedded steel reinforcement bar (Ø = 6 mm) were casted for self-healing experiments. All the mortar specimens were prepared by following the standard procedure described in EN 196-1. Plain control mixture was composed of DIN EN 196-1 standard sand (1350 g), CEM I 42.5R cement (450 g) and, tap water (225 g) with a weight ratio of 3:1:0.5. In addition to the typical aforementioned structural ingredients (i.e. sand, cement, water), abiotic control mixture contained 5.00% CF w/w cement and 2.00% CN w/w cement, which was determined based on a previous study [24]. In biomortar specimens, together with the chemical admixtures CF and CN, dry ACDC granules (1.45% w/w cement) were added into the mix as self-healing agent. The latter corresponds to addition of bacteria at a dose of 1.00 % w/w cement as 70% of the biogranule content is bacteria. The incorporation was done without using any additional protective carrier as ACDC is a self-protected granulated culture that can survive concrete incorporation in the absence of protective carriers [8].

In the biomortar mix, 50% w/w of the incorporated biogranules were between 1.00 to 2.00 mm in size, 35% w/w of the incorporated biogranules were between 0.85 to 1 mm in size and 15% w/w of the incorporated biogranules were between 0.45 to 0.85 mm in size.

Upon demoulding, specimens were cured for 28 days at room temperature inside a tightly sealed bag. Cured specimens were cracked by applying uniaxial tension to the embedded rebar at a speed of 0.01 mm/s under stroke control. Multiple cracks with crack widths ranging between 50 – 600 µm were achieved in all mortar specimens.

Self-healing performances of the cracked specimens were investigated under weekly alternating wet-dry conditions (i.e. 1 week wet, 1 week dry treatment). A climate chamber was set to >90% relative humidity and 20°C for dry treatment.

2.3.2. Quantification of the self-healing performance of mortars

Healing was monitored weekly under a stereomicroscope and quantified for cracks with 50–600 µm crack width. During microscopic analysis, specimens exposed to ambient air conditions (~20°C). An image analysis software was used to analyse the obtained micrographs and quantify the healing efficiency. Healing efficiency was calculated by using Equation (7).

$$\text{Healing Efficiency } \% = \left[1 - \frac{w_t}{w_{initial}}\right] \times 100 \tag{7}$$

where w_t is crack width measured at a certain time t(d) and $w_{initial}$ is the initial crack width.

2.3.3. Water tightness of the healed cracks

Water tightness regain of the healed specimens was quantified by conducting capillary sorption tests on 255±80 µm wide crack. Prior to testing, the specimens were dried in an oven at 40°C until the mass changes in 24 h were less than 0.1%. Similar crack widths were chosen for each type of specimen. Apart from the chosen crack the rest of the specimen was completely covered with aluminum tape to have localized water ingress around the crack. Therefore, only the area of 3 cm^2 (30 × 10 mm) surrounding the chosen crack was in contact with the water throughout the test. The mass increase of specimens due to the absorbed water was monitored in regular time intervals for 52 hours. A wet towel was used to remove the remaining surface water droplets prior to weighing. Water tightness regain was quantified relative to water tightnesses of autogenously healed plain and abiotic control specimens.

2.3.4. Data interpretation and statistical analysis

The significance of variation between self-healing performances (either crack closure or water tightness regain) of the investigated mortar types was analysed by means of one way ANOVA test (p=0.05) using SigmaPlot v12.0 (Systat Software Inc., USA) software.

3. RESULTS AND DISCUSSION

ACDC biogranules were obtained successfully and quality assessment revealed that the properties were similar to those produced in previous studies (data not shown, can be found in [13]). Obtained granules are visually represented in Figure 1.

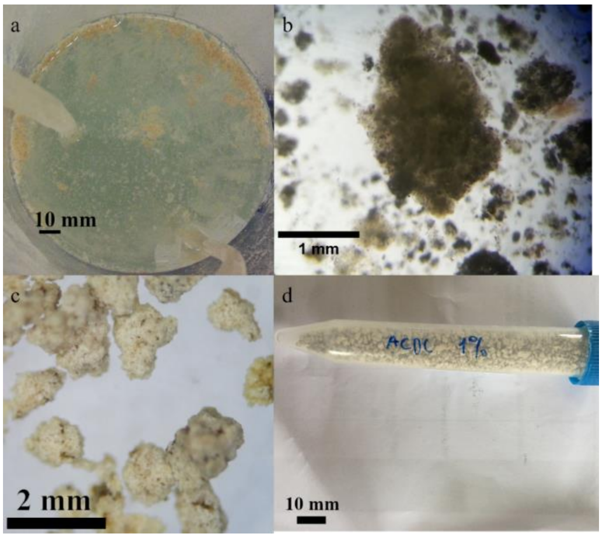

Figure 1. Appearance of biogranules produced in sequencing batch reactor, inside the reactor (a); under microscope before drying (b); under microscope after drying (c); packed and stored for application (d).

The wet particle sizes of ACDC biogranules in the bioreactor varied between 0.21 mm to 4.50 mm. About 10% of the bioreactor content was floccular sludge (data not shown). After drying the ACDC biogranules only the fraction with particle sizes varying between 0.45 mm to 2.00 mm were used which corresponded to the 66% of the harvested total.

3.1. Enhanced self-healing under alternating wet/dry treatment

Figure 2 represents the weekly self-healing performances of plain control, abiotic control and microbial self-healing mortar containing 1.45% ACDC biogranules (1.00% bacteria) w/w cement. Different from the conventional fresh water immersion treatment, wet/dry treatment was investigated in this study. At the end of four weeks treatment, significant difference (*p*=0.05) was observed between the crack closure performances of microbially self-healing and autogenously self-healing specimens. Autogenous healing limit for both plain and abiotic control specimens were determined as around 150 µm and 100 µm, respectively, while the limit for microbial self-healing was a little bit higher than 400 µm with few partially healed cracks of 100 to 200 µm wide (Figure 2c, Table 2). Yet, the number of partially healed

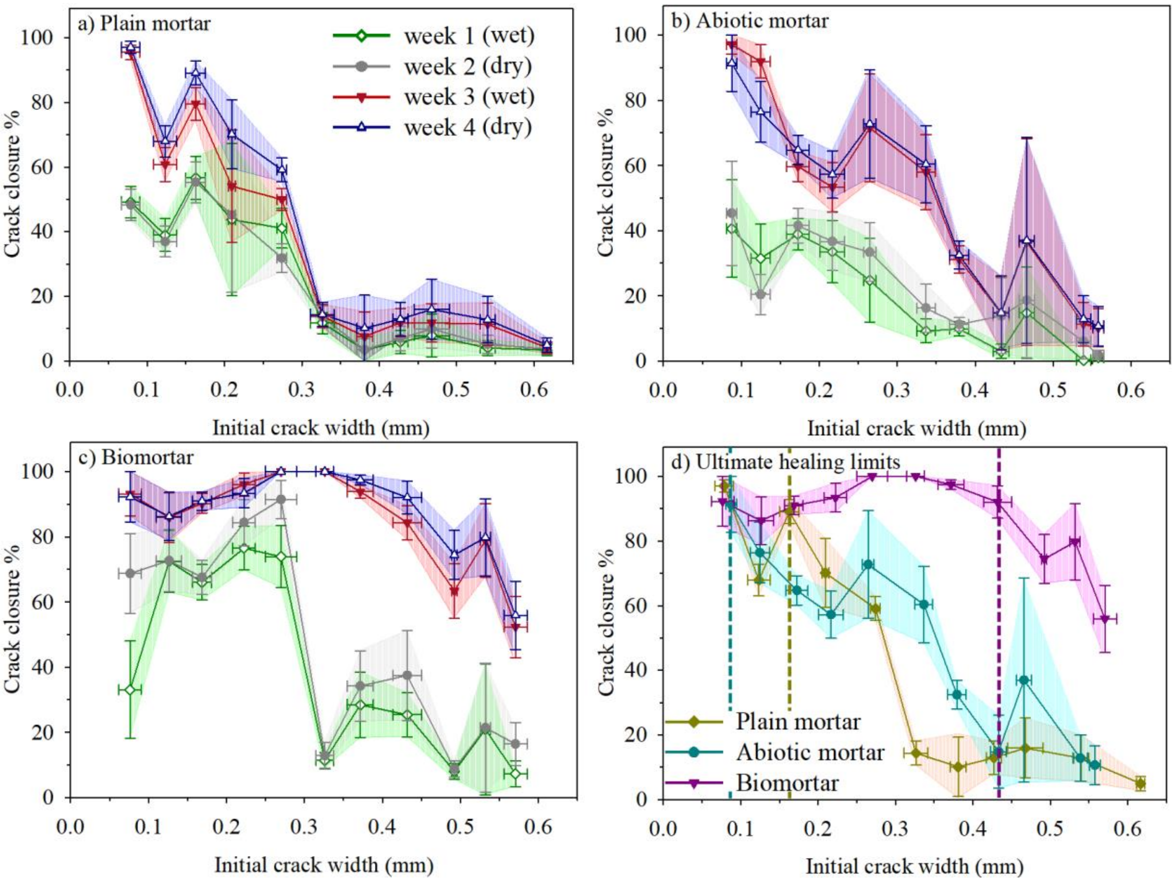

Figure 2. Biogranule containing biomortar reveals enhanced healing properties under weekly alternating wet/dry conditions when weekly average crack closure percentages in plain mortar (a); abiotic mortar (b); 1.45% ACDC containing biomortar (c); and ultimate healing limits at the end of the treatment period (d) are compared. Horizontal error bars represent the standard deviation, crack widths were grouped with 50µm intervals, vertical error bars represent the standard error of the mean, n ≥ 10. For interpretation of the references to colours in this figure legend, the reader is referred to the web version of this article.

Table 2. Enhanced healing performance of biomortar in consecutive weeks of the treatment period

Type	Maximum healed crack width* (μm)			
	1st week (wet)	2nd week (dry)	3rd week (wet)	4th week (dry)
Plain mortar	NA	NA	79±12	163±13
Abiotic mortar	NA	NA	125±13	88±6
Biomortar	NA	270±20	372±17	432±18

*For detection of maximum healed crack width, 90% crack closure was used as a threshold.

cracks were fewer when compared to the control specimens (Figure 2a,b). Therefore, even in the worst-case scenario, microbial self-healing mortar still performed better than any of the control mortars.

The results indicated a major outcome that the wet treatment time is the critical parameter for microbial self-healing performance (Table 2). Presence of humidity contributed only 10% of the overall crack closure performance (Figure 2a,b,c, Table 2). In the same treatment period (four weeks), variation in crack healing was much higher in this study, when compared to previously reported results on crack closure performance of completely immersed self-healing specimens [2,18]. The inefficient healing at dry periods showed consistency with the previous findings. Wang et al. [9,17,22] reported for ureolytic bacteria that microbial self-healing did not occur at both 60% RH and 95% RH treatments. Therefore, it can be said that, regardless of the metabolic pathway, dry or humid periods have no direct influence on self-healing of concrete cracks. Their indirect influence on the overall healing performance is discussed in the following parts of this article.

It should also be noted that the autogenous healing limits achieved in this study were 100 to 150 μm lower than the ones (200 – 250 μm) reported for similar mixtures upon continuous fresh water immersion [2,7]. The relatively low autogenous healing performances achieved in this study was attributed to the inefficient contribution of dry periods on formation of healing products. Autogenous healing limit plays a significant role in the ultimate healing limit of biomortar specimens [2]. A decrease in autogenous healing limit due to a certain reason cause a decrease in the ultimate healing limit of biomortar specimens as well [2]. Accordingly, in this study, for ACDC containing biomortar, the microbial healing limit achieved upon 4 weeks of wet-dry cycle treatment was ~400 μm. In a previous study, where identical amount of ACDC was used, the microbial healing limit upon 4 weeks of fresh water immersion was determined as ~500 μm [2] which was 100 μm more than the one achieved in this study. The difference between the microbial healing limits of the two studies shows consistency with the aforementioned difference (100 – 150 μm) between the autogenous healing limits achieved upon different treatments. Therefore, it can be claimed that the dry periods have more severe impact on autogenous healing than the microbial healing which negatively affected the ultimate healing limit. It should be noted that fresh water immersion was applied to ensure the wet periods in this study. Some studies revealed that the autogenous healing limit can reach up to 600 μm if the specimens are immersed in sea water [25]. Therefore, it is possible to achieve a higher ultimate healing limit under wet/dry conditions if sea water immersion is used instead of fresh water immersion to ensure the wet periods of the wet/dry cycles.

Considering that the dry periods have little contribution to crack closure performance (Figure 2c,d), 4 weeks of alternating wet/dry treatment should correspond to about two weeks of fresh water immersion in other studies [17,18], and the obtained self-healing performances can be interpreted accordingly to unveil the possible indirect effects of dry periods on self-healing. Previously reported autogenous healing limits for two weeks fresh water immersion period were between 100 to 200 μm [7,18] which were in consistency with our observations and, particularly for autogenous healing, confirm our assumption that 4 weeks of alternating wet/dry treatment corresponds to about two weeks of fresh water immersion.

Wang et al. [17] presented that in biomortar specimens containing microencapsulated ureolytic spores, only around 50% of the ~400 μm wide crack could be healed in the first two weeks of the fresh water immersion. Silva [26] investigated the healing performances of different biomortar specimens containing diatomaceous earth (DE) immobilized *Bacillus sphaericus*, DE immobilized *Bacillus cohnii* and a cyclic enriched non-axenic ureolytic powder as microbial healing agents and after 2 weeks of fresh water immersion, the healing limits were defined as 200 μm, 200 μm and 100 μm, respectively [26]. Also in a previous study of the author [18], where expanded clay immobilized nitrate reducing bacteria, namely *Pseudomonas aeruginosa* and *Diaphorobacter nitroreducens* were tested as healing agents, it was reported that *Pseudomonas aeruginosa* containing biomortar specimens could heal only 200 μm crack in the first two weeks of the fresh water immersion. Although *Diaphorobacter nitroreducens* containing biomortar specimens could heal a wider crack width (~300 μm) in the same period of time, the healing was not consistent among different cracks with the same width to claim a precise healing limit. Comparing the findings of this study with the outcomes of aforementioned previous studies revealed the actual effect of a week-long dry periods between the two wet periods. It can be claimed that a better healing efficiency could be achieved by coupling fresh water immersion treatment with a week-long dry or humid period between two wet periods. This enhanced performance could be attributed to the physicochemical influences of dry periods on microbial induced calcium carbonate precipitation. It is known that MICP takes place in the microenvironment of the corresponding bacteria and the concentration of ions in the microenvironment plays an important role in MICP rate [4]. Increasing ion concentration in the microenvironment of the cells due to microbial activity results in calcium carbonate precipitation. The water evaporation during the dry periods could also increase the concentration of all the ions in the microenvironment and thus accelerated the precipitation of calcium carbonate minerals. The positive influence of dry periods on crack healing performance was only significant in biomortar specimens (Figure 2c) which confirmed the impact of wet/dry cycles on MICP. Therefore, it can be concluded that although dry periods have no direct positive influence on microbial activity, they indirectly enhance the formation of calcium carbonate crystals and thus slightly improve (~10%) crack healing efficiency.

Considering the aforementioned discrete influences of dry periods on autogenous healing and microbial healing, it should be noted that dry periods negatively affect the autogenous healing and positively affect the microbial healing. Since the negative effect on autogenous healing is more dramatic when compared to its positive effect on microbial healing, the overall healing performance of biomortar under wet-dry cycles treatment became less efficient than its performance under fresh water immersion for an identical treatment duration of 28 days. For fresh water immersion, it was reported that the healable crack width increases as the treatment duration increases [14,17,18]. Therefore, by increasing the number of wet/dry treatment cycles, one can improve the ultimately healable crack width limit defined in this study. Moreover, the contribution of the autogenous healing to the overall healing decreases as the cementitious material ages [2]. Therefore, wet-dry cycle treatment might lead to better healing efficiencies on cracks occurring in aged concrete, which requires further investigation. As mentioned previously, the environmental conditions creating the wet conditions (fresh water or sea water) play a significant role in the autogenous healing limit as well. Therefore, in marine conditions, where spraying and wet/dry cycles are prevalent, one may expect a higher ultimate healing limit for the tested biomortar specimens which also requires further investigation.

Autogenous healing in concrete is highly variable in rate, constituent dependent and unpredictable which cause large variations in crack healing performances recorded at early periods of the healing process, particularly in microbial self-healing concrete. As the treatment duration increases, variation in crack healing performance decreases, since the interference of the variations in autogenous healing to the overall healing becomes less significant [14,17,18]. Previous studies revealed that during fresh water immersion of the cracked specimens, in the first 2 weeks, it was hard to distinguish between control and bacteria-based specimens [16,18,22]. However, in this study, at the end of 3 weeks alternating wet/dry treatments (2 weeks of wet period), the enhanced self-healing performance of bacteria-based concrete could be distinguished clearly (Figure 2). This was attributed to the inverse effect of dry periods on autogenous healing and microbial healing. Dry periods negatively influenced the autogenous healing performance and positively influenced the microbial healing performance, thus the difference in healing performances of control and microbial specimens became more noticeable in just three weeks.

3.2. Recovery of water tightness upon microbial self-healing of cracks

Upon crack healing tests, cracks presented in Figure 3 were tested for capillary water absorption. Capillary water absorption through the healed cracks of biomortar specimens were 44% less than the autogenously healed control specimens (Figure 4). The difference was attributed to the sealing effect of the calcium carbonate minerals formed at the crack mouth (Figure 3c). As reported previously, microbial self-healing of concrete cracks mostly occurs at the crack mouth and provides a sealing effect [22].

The thickness of the sealing layer is an important factor for decreasing the permeability of the cracks [14,18]. The thickness of the sealing layer was not quantified in this study, but comparative assessment was made. Studies clearly depicting the treatment type, microbial and autogenous healing efficiencies, sealing layer thickness and the investigated crack widths were used during the comparative assessment. Collected relevant information for comparative assessment is summarized in Table 3.

In our previous study, where axenic nitrate reducing cultures were tested as healing agents, autogenous healing for a 235±35 μm wide crack was recorded as 90% (Table 3). Water absorption of completely healed biomortar specimen was 40% less than the autogenously healed control specimen and the thickness of the sealing layer leading to the reported difference was between 5 to 13 mm (Table 3). In another study, ACDC biogranules were used as healing agent to heal a 399±27 μm wide crack via fresh water immersion. For the corresponding crack width autogenous crack healing efficiency was recorded as 40% and the permeability of completely healed biomortar specimen was 70% less than the autogenously healed control specimen (Table 3). The thickness of the sealing layer leading to the reported difference was between 3 to 10 mm (Table 3). In another study, where ureolytic *Bacillus sphaericus* culture was used as microbial healing agent and the healing was triggered via wet-dry cycles, crack widths ranging between 200 – 220 μm could be completely healed by bacteria (Table 3). The autogenous healing was ~55% for the corresponding crack width range and the water

Table 3. Relevant information used for comparative assessment of sealing layer thickness in healed biomortar specimens

Studies	Microbial pathway	Treatment type	Crack width (μm)	Control healing	Water tightness of biomortar*	Sealing layer thickness in biomortar
Ersan et al. [18]	Nitrate reduction	Fresh water immersion	235±35	90%	40% (+)	5 – 13 mm
Ersan et al. [14]	Nitrate reduction	Fresh water immersion	400±25	40%	70% (+)	3 – 10 mm
Wang et al. [9,22]	Urea hydrolysis	1h wet – 11h dry	210±10	55%	30% (+)	1 – 3 mm
This study	Nitrate reduction	1w wet – 1w dry	255±80	65%	44% (+)	3 – 5 mm

* (+) sign for water tightness regain represents percent better water tightness regain compared to autogenously healed control specimen.

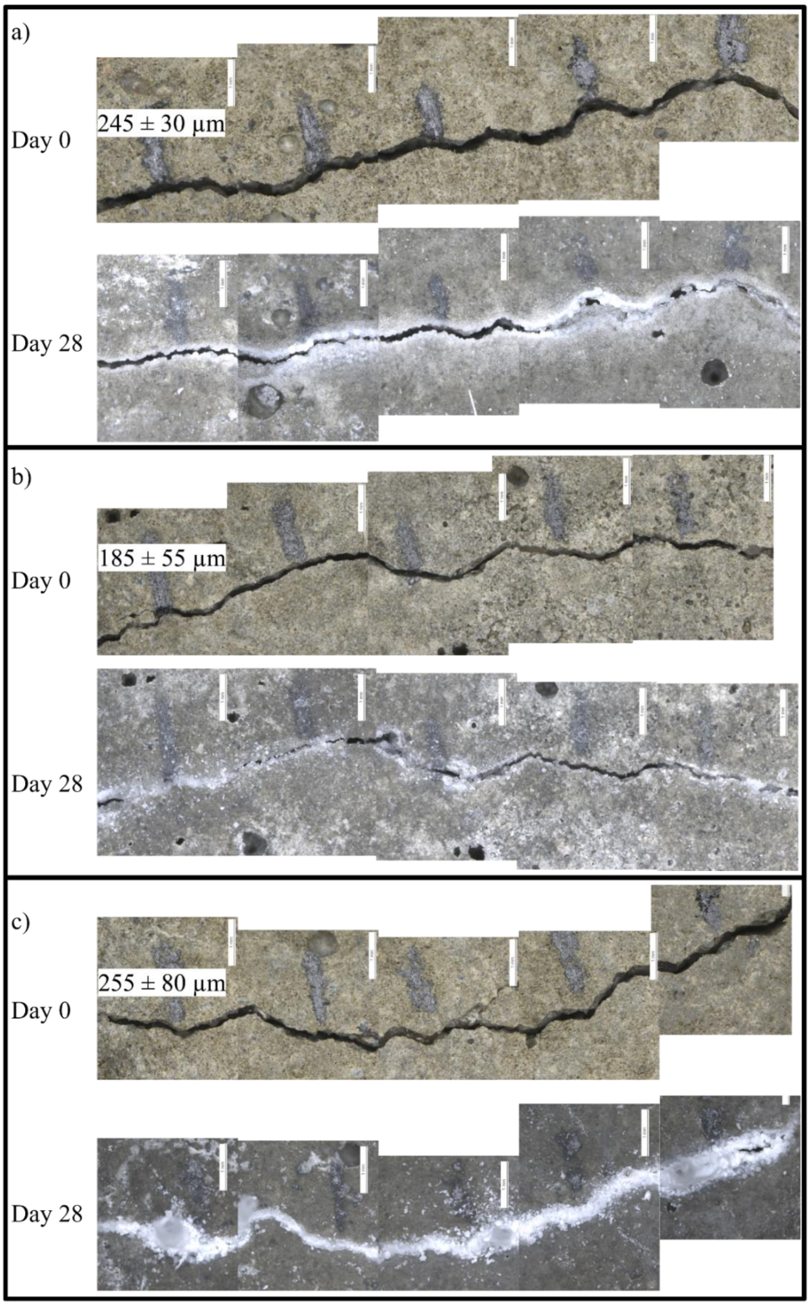

Figure 3. The micrographs showing the initial (before treatment) and the final (after 4 weeks of wet/dry treatments) appearance of the cracks in (a) plain mortar specimen (b) abiotic control specimen (c) biomortar specimen which are also tested for capillary water absorption. White scale bars in each micrograph are identical and represent 1 mm.

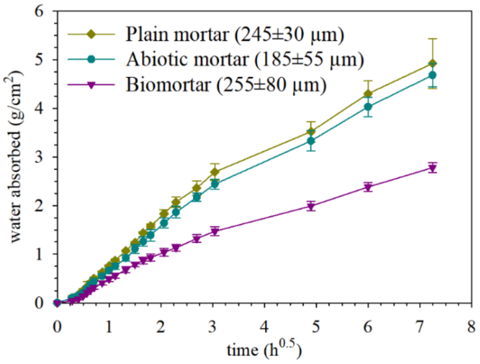

Figure 4. Enhanced water tightness regain of microbially healed biomortar specimens over autogenously healed control specimens. Error bars represent the standard deviation (n=3).

tightness regain of the biomortar specimens were 30% better than the autogenously healed control specimens (Table 3). The thickness of the sealing layer in healed biomortar specimen was reported to be between 1-3 mm (Table 3). In this study, the autogenous healing efficiency of 245±30 µm wide cracks was around 65% in plain control specimens (Figure 2d). The capillary water tightness of 255±80 µm wide crack in biomortar was 44% better compared to the autogenously healed control specimen (Figure 4). Comparative evaluation revealed that on the one hand, the water tightness achieved upon microbial healing of cracks via wet/dry cycles treatment, is slightly less than those achieved via fresh water immersion for a similar crack width (Table 3). On the other hand, for a similar crack width range, when healing is triggered via wet-dry cycles, nitrate reducing bacteria provides slightly better water tightness regain than ureolytic bacteria. Based on this comparison, it is safe to claim that in this study, the thickness of the sealing layer in biomortar specimens would be in the range close to the lower boundary of those reported for fresh water immersion treatment (3 – 5 mm). It is also safe to claim that the thickness of the sealing layer would be higher than the upper boundary of that reported for urea hydrolysis pathway and wet-dry cycle treatment (> 3 mm). Accordingly, the sealing layer thickness in this study appeared to be between 3 to 5 mm (Table 3).

4. CONCLUSION

ACDC containing biomortars could self-heal crack widths as wide as 400 µm upon intermittent week-long wet/dry treatments for four weeks, while control specimens could only heal up to 150 µm wide cracks in the same period.

Dry periods have severe negative impact on autogenous healing performance as they slow down formation of healing products while they have slightly positive contribution to the microbial self-healing by increasing the concentration of ions in the microenvironment of bacteria and enhancing the calcium carbonate precipitation rate.

The major limiting factor for the ultimate healing limit of microbial self-healing concrete appeared to be the poor autogenous healing performance in dry periods rather than the ceased microbial activity.

In a week-long wet/dry cycle treatment, dry periods contribute to only 10% of the ultimate healing performance of biomortars, and hence complete healing of a certain crack width requires more time compared to the healing times noted in literature for continuous immersion treatment.

Due to the difference in healing performances of ~250 µm wide cracks in biomortar and traditional mortar, biomortars enable 44% better water tightness regain than traditional mortar specimens.

Since biogranule containing self-healing concrete reveals decent healing performance under wet/dry cycles, it can be used not only in completely immersed structures but also in structures where spraying or intermittent wetting occurs. The latter enables the use of microbial self-healing concrete in broader range of applications.

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DECLARATION OF ETHICAL STANDARDS

The authors of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

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