INVESTIGATION OF PERFORMANCE PROPERTIES OF CEMENT-BINDER SYSTEMS WITH MICROBIAL SELF-HEALING ABILITY

MİKROBİYAL KENDİLİĞİNDEN İYİLEŞME KABİLİYETİNE SAHİP ÇİMENTO BAĞLAYICILI SİSTEMLERİN PERFORMANS ÖZELLİKLERİNİN İNCELENMESİ

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ABSTRACT

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Microbials incorporated cement-binder system is a kind of self-healing cementitious material that contains micro-organisms which induce a precipitation of calcium carbonate in cracks. Ureolytic pure cultures have been used in the majority of research conducted for microbials incorporated cement-binder system development. Biogranules made up of nitrate-reducing bacteria have lately become more sustainable, cheaper, and feasible. Since in the self-healing studies of microbial introduced cement binding systems the bacteria content was selected randomly, it is still unknown the most acceptable content of bacteria for self-healing in these systems. In this thesis study, the biogranules , which had 0.45 to 2.00 mm of size and have an active nitrate-reducing core surrounded by a protective outside layer consisting of $CaCO_3$ and $Ca_3(PO_4)_2$, were produced in the laboratory and, acceptable biogranules content for self-healing cement-binder systems was investigated. During the assessment of the acceptable content of biogranules, cement-binder systems with various doses of biogranules were analyzed with regards to their workability, compressive strength, self-healing rate, and self-healing. Mortar specimens with 12 different doses of biogranules corresponding to the contents of bacteria ranging from 0.05% to 2.50% w/w cement were poured and cracks were obtained at the end of a 28-day curing period. The cracked specimens were held in tap water over 4 weeks and

weekly percentages of crack closure have been monitored to investigate the acceptable and minimum levels of bacteria for microbial cement binding systems by using macroscope. The addition of bacteria into the mortar in the form of biogranules up to 2,50% w/w cement has no adverse effect on the properties of the mortar. The acceptable and lowest bacteria content in microbial self-healing concrete should be 0.25% and 0.05% w/w cement, respectively, based on microbial self-healing rates and capacities when added in the form of biogranules. Cracks as wide as 800 ± 30 μm could be healed in acceptable doses after 3 weeks, but this healing performance decreased with an increase or decrease in the bacteria content. Microbial healing performed consistently better than autogenous healing independent of bacteria content.

Keywords: Self-healing concrete, bacteria content, biogranule, nitrate reducing bacteria, self-protected culture

ÖZET

MİKROBİYAL KENDİLİĞİNDEN İYİLEŞME KABİLİYETİNE SAHİP ÇİMENTO BAĞLAYICILI SİSTEMLERİN PERFORMANS ÖZELLİKLERİNİN İNCELENMESİ

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Mikrobiyal katkılı çimento-bağlayıcı sistem, çatlaklarda kalsiyum karbonat çökelmesine neden olan mikroorganizmaları içeren kendi kendini iyileştiren bir tür çimentolu malzemedir. Üreolitik saf kültürler, çimento-bağlayıcı sistem geliştirme dahil mikrobiyaller için yürütülen araştırmaların çoğunda kullanılmıştır. Nitrat azaltan bakterilerden oluşan biyogranüller son zamanlarda daha sürdürülebilir, daha ucuz ve uygulanabilir hale gelmiştir. Mikrobiyal katkılı çimento bağlama sistemlerinin kendi kendini iyileştirme çalışmalarında bakteri içeriği rastgele seçildiğinden, bu sistemlerde kendi kendini iyileştirme için en kabul edilebilir bakteri içeriği hala bilinmemektedir. Bu tez çalışmasında, laboratuvar ortamında $CaCO_3$ ve $Ca_3(PO_4)_2$'den oluşan koruyucu bir dış katmanla çevrili aktif nitrat azaltıcı çekirdeğe sahip 0,45 ile 2,00 mm boyutlarında biyogranüller üretilmiş ve kabul edilebilir biyogranül içeriğine sahip biyogranüller üretilmiştir ve kendini onaran çimento-bağlayıcı sistemlerde kullanılabilecek kabul edilebilir biyogranül miktarı incelenmiştir. Biyogranüllerin kabul edilebilir miktarının değerlendirilmesi sırasında, çeşitli dozlarda biyogranül içeren çimento-bağlayıcı sistemler, işlenebilirlik, basınç dayanımı, kendi kendini iyileştirme hızı ve kendi kendine iyileşme açısından analiz edilmiştir. Çimento ağırlığınca %0,05 ile %2,50 arasında değişen oranlarda biyogranül formunda bakteri içeren 12 farklı harç numuneleri

hazırlanmıştır ve 28 günlük kür süresi sonunda çatlaklar elde edilmiştir. Çatlak numuneler 4 hafta boyunca musluk suyunda tutulmuş ve mikrobiyal çimento bağlayıcılı sistemler için kabul edilebilir ve minimum bakteri seviyelerini incelemek için haftalık çatlak kapanma yüzdeleri makroskop kullanılarak izlenmiştir. Harca biyogranül formunda çimento ağırlığınca %2,50'a kadar bakteri eklenmesi harcın özelliklerine olumsuz etkide bulunmamıştır. Mikrobiyal kendi kendini iyileştiren çimento bağlayıcı sistemde kabul edilebilir ve en düşük bakteri içeriği, biyogranül şeklinde eklendiğinde mikrobiyal kendi kendini iyileştirme oranları ve kapasitelerine göre sırasıyla, çimento ağırlığınca %0.25 ve %0.05 olarak belirlenmiştir. 800 ± 30 μm genişliğindeki çatlaklar, 3 hafta sonra kabul edilebilir dozlarda iyileştirilebilmiş, ancak bu iyileşme performansı, bakteri içeriğindeki artış veya azalma ile azalmıştır. Çimento bağlayıcılı sistemde, mikrobiyal iyileşme, bakteri içeriğinden bağımsız olarak otojen iyileşmeden tutarlı bir şekilde daha iyi performans göstermiştir.

Anahtar kelimeler: Kendini onaran beton, bakteri miktarı, biyogranül, nitrat indirgeyen bakteri, kendini koruyan kültür

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MERVE SÖNMEZ
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SYMBOLS AND ABBREVIATIONS

Abbreviations

ACDC	Activated Compact Denitrfiying Core
Bio-0.05%	Mortar with biogranules at the rate of 0.05% by weight of cement
Bio-0.10%	Mortar with biogranules at the rate of 0.10% by weight of cement
Bio-0.25%	Mortar with biogranules at the rate of 0.25% by weight of cement
Bio-0.50%	Mortar with biogranules at the rate of 0.50% by weight of cement
Bio-0.75%	Mortar with biogranules at the rate of 0.75% by weight of cement
Bio-1.00%	Mortar with biogranules at the rate of 1.00% by weight of cement
Bio-1.25%	Mortar with biogranules at the rate of 1.25% by weight of cement
Bio-1.50%	Mortar with biogranules at the rate of 1.50% by weight of cement
Bio-1.75%	Mortar with biogranules at the rate of 1.75% by weight of cement
Bio-2.00%	Mortar with biogranules at the rate of 2.00% by weight of cement
Bio-2.25%	Mortar with biogranules at the rate of 2.25% by weight of cement
Bio-2.50%	Mortar with biogranules at the rate of 2.50% by weight of cement
Bio-3.00%	Mortar with biogranules at the rate of 3.00% by weight of cement
Bio C-0.25%	Concrete with biogranules at rate of 0.25% by weight of cement
Bio C-2.50%	Concrete with biogranules at rate of 2.50% by weight of cement
C	Abiotic control specimen
Bioconcrete	Bacteria incorporated cement-binder system
Biomortar	Bacteria incorporated mortar
$CaCO_3$	Calcium carbonate
CERUP	Cyclic Enriched Ureolytic Powder
CF	Calcium formate
CN	Calcium nitrate
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
ECC	Engineered Cementitious Composites
EDS	Energy Dispersive X-Ray Spectroscopy
EPA	Environmental Protection Agency
FTIR	Fourier Transform Infrared Spectroscopy

MICP	Microbially Induced Calcite Precipitation
R	Reference mortar specimen
SAP	Superabsorbent Polymers
SBR	Sequential Batch Reactor
SEM	Scanning Electron Microscopy
SRB	Sulfate Reducing Bacteria
SVI	Sludge Volume Index
TN	Total Nitrogen
TSS	Total Suspended Solids
VSS	Volatile Suspended Solid

1. INTRODUCTION

Cementitious composites, which were first used in the Ancient Roman period, have taken their place among the popular building materials in the construction sector since then and are still the most widely used building materials today. Concrete is a cementitious composite which is composed of a binder and filler agent [1], [2]. Among cement composites, the most widely used are concrete materials. Considering the global concrete production, annual concrete production per person is around 4.7 tons. Its low cost, good fire resistance and high compressive strength make concrete unrivalled in the industry with annual consumption of 33 billion tons per year [3]. On the other hand, the biggest drawbacks of concrete materials are demonstrating low strength under tensile stresses and the high CO_2 footprint.

Concrete production is responsible for 8% of global carbon dioxide (CO_2) emission due to cement production. During cement production, clay and limestone is heated to 1500℃ to produce clinker. For heating, a significant amount of energy generated from fossil fuel combustion[4]. Emitted CO_2 during calcination which is the decomposition of $CaCO_3$ into CaO and CO_2, is about 60% of total emission and rest emission is generated by fossil fuels combustion to heat raw materials [5]. Despite economic effects of the COVID-19 pandemic, the world annual cement production did not decrease significantly as surpassing 4 billion ton in 2019 [6]. EPA reported that for one ton of cement production 900 to 1100 kg CO_2 releases [5]. Cement production was responsible for 4% of the global CO_2 emission in 2019 and emissions from cement rose by 2.1% compared to 2018 [7]. Increasing sensitivity to the environment made it possible to draw attention to the optimized concrete mixes, the term "green concrete" and recycling-based composite materials to reduce the CO_2 emission generated during concrete production [8]–[10].

Another drawback of concrete is displaying low strength under tensile loads. The deformation capacity of a standard concrete under axial tensile stress corresponds to approximately 10% of the deformation it shows under pressure [11]. This problem has been solved by using steel reinforcing bars or different types of fibers (steel, polypropylene, glass, carbon, organic). The most used reinforcement tool until now is steel reinforcements. Since the main element carrying tensile loads is reinforcements, the

durability of the structure depends on the condition of the reinforcement. An alkaline environment in concrete helps to create passivation of the steel surface however it is not sufficient in order to maintain reinforcements in good condition during service [12]. Steel reinforcements are exposed to environmental conditions due to cracking which is the most happened problem in concrete, by increased permeability. Carbonation of concrete is facilitated by this exposure and the pH decreases accordingly. This situation, which also prevents passivation of the reinforcement, allows chloride ingress with increased permeability, and then corrosion and related durability problems arise in the reinforcement.

1.1. Concrete Cracks

Concrete shows low resistance under tensile load and it behaves non-linear. Concrete beams are often unable to bear the axial stress caused by even their own weight, and cracks are observed. For this reason, concrete can be named as a semi-brittle material with a tendency to crack. As aforementioned, in order to compensate for this deficiency, structural concretes are reinforced with steel reinforcements, and strengths that can meet service conditions under axial stress are obtained. Although concrete materials reinforced with steel reinforcements can be used for structural purposes, micro-cracks on concrete due to mechanical, physical, and chemical reasons cannot be prevented. Serious cracks in concrete are often observed when it is subjected to tensile stresses which are higher than its tensile strength. Tensile stress can be caused by detrimental reactions, external attacks, and mechanical loading. Moreover, there can be many other causes of cracks. The most common concrete cracks can be classified into two main types: Cracks in plastic concrete and cracks in hardened concrete. Reasons for plastic concrete cracks can be plastic or autogenous shrinkage, formwork or sub-grade movement and freezing, etc. The reasons for cracks in hardened concrete can be thermal changes in hydration and, autogenous or drying shrinkage, etc. at the early age. In aged concrete, corrosion of rebars, fatigue, freezing/thawing, drying shrinkage, and overload can be reasons for cracks [13].

1.2. Crack Related Durability Issues

Throughout their lives, structures are subjected to chemical and physical attacks, and the permeability of concrete plays a significant impact in the amount of damage sustained. The structure becomes more responsive the more permeable the concrete is. The existence of cracks, in particular, enhances the permeability of concrete and aggravates steel reinforcement degradation. When the crack width exceeds the critical limit, it becomes a severe problem in terms of durability, aesthetics, and serviceability. Because the critical crack width is highly dependent on intrinsic and extrinsic factors, there is no exact value that can be used as a constraint. The allowable crack width is however regarded as 0.41 mm in ACI 318-95 and earlier. Furthermore, definite limits are recommended for ACI 224R-01 [14] and EN 1992-1-1 [15], which take account of various conditions of exposure and autogenous healing properties. The experimental values showed that the level of entry of chloride into cracked, uncracked concrete was only similar in comparison to those suggested values when its crack splitting was less than 10 μm [16].

Table 1. 1. Plausible crack widths for reinforced concrete when autogenous healing is taken into account.

Exposure conditions	**Crack width (μm)**		
	ACI-224R[14]	**EN-1992-1-1**[15]	
	Service load	**Quasi-permanent load**	**Frequent load**
De-icing chemicals	180	300	200
Humidity, moist air, soil	300	400	200
Dry air or protective membrane	410	400	200
Water retaining structures except non-pressure pipes	100	300	200
Seawater and seawater spray, wetting and drying	150	300	200

Concrete cracks are brittle and typically form as a result of environmental factors. The most important mechanisms in crack development may be identified as freeze thaw, external chemical attack (Cl^-, SO_4^{2-}), intrinsic chemical reactions (e.g. corrosion, alkaline-silica reactions)[14]. Repairing concrete cracks is therefore of importance in order to keep the structure healthy and prevent problems with durability. Thus far, either epoxy resin or various strategies have been applied in the development of self-healing concrete materials and work on new strategies continues at full speed. polyurethane foam is the most commonly used way of repairing the crack. Unless proper maintenance is performed, reinforcing begins to corrode and structural failure becomes unavoidable. There are considerable researchs in order to find alternative ways of solving problems of durability and reducing maintenance costs. One of the strategies is to develop self-healing concrete.

There are different self-healing agent and methods utilized to enhance crack closure. In a study, specimens with different micro-cracking dimensions were exposed for 1, 2, or 3 months to a 3 percent chloride concentration solution and then reloaded until residual tensile properties have not been measured. Measuring the retained rigidity, ultimate tensile strength, tensile stress capacity, and crack width was evaluated by the effect of cracking and self-healing within ECC under combined mechanical loading and exposure to chloride. The effect of exposure to chloride on the ECC matrix and interfacial properties of fiber / matrix were also investigated. This study shows that ECC is capable of self-healing crack damage in an environment of high chloride levels. In ECC, which has had up to 1.5 percent of tensile strain charged and submerged in 3% NaCl solution for 30 days or more, almost complete healing for material rigidity and self-healing capacity was discovered in a water environment similar to autogenous healing[17].

Moreover, in comparison with the superabsorbent polymers (SAPs)-free samples, mortar samples containing have a low overall uptake in a crack. Due to their inflating capacity, SAP particles reduce moisture intake in a crack. Thus, the structure is penetrated to a lesser extent by harmful substances dissolved in fluids. As a result, the SAP particles are able to retain the fluid within their structure, and the crack closes on its own[18]

The microscopic analysis of the pre-loaded ECC samples has shown that self-healing, whatever the mixture, has been achieved up to certain limits. Nevertheless, the main

limitation for ultimate self-healing was linked to the allowed crack widths. F_ECC (mixture with Class-F fly ash) specimens were reported to be able to heal the cracks with 30 μm of width. For S_ECC (mixture with slag) specimens this was approximately 50 μm for C_ECC (mixture with Class-C fly ash) and just over 100 μm. The microstructural analysis also revealed that the major self-healing products are calcite and C–S–H gels, which vary depending on ECC mixtures with different supplementary cementitious materials (SCM) types [19].

Mignon et al. study revealed that a variety of (superabsorbent) polymers (SAP), formed of acrylic acid and methylene bizacrylamide as cross-linker, have been produced and classified in previous research. On the basis of these findings, two SAPs of greatest quality were selected for further characterisation and were associated with the possible self-sealing and self-healing of concrete cracks. Bending and compression tests in morter demonstrate that more SAP leads to a greater reduction in strength (up to 52 percent loss of compression strength). On the other hand, the addition of greater SAP amounts in comparison with reference examples without SAP leads to a better self-sealing effect[20] In another study, four methods of self-healing were utilized independently as well as in combination. These included: (1) using the University of Cambridge-developed microcapsules containing mineral healing agents in conjunction with industry; (2) bacterial healing dependent on the knowledge of Bath University; (3) the use of a crack-closing shape memory polymers (SMP) system; and (4) the supply of a mineral healing agent through the vascular system [21].

Joseph et al. study showed that the final five pairs of tests used a supply system with capillary tubes accessible to the surrounding air. These tests explored the impacts of the adhesive healing reaction within the mortar beams and its effect on this self-healing reaction, reinforced level and load rate. The first and second loading cycle were demonstrated with both main and secondary healing responses [22].

2. LITERATURE REVIEW

2.1. Self-healing of Concrete

In terms of increasing sustainability, it is important that reinforced concrete structures are more durable and have a longer life. In recent years, researchers have focused on the development of concrete materials that can repair their own cracks in order to prevent the problems caused by cracks in reinforced concrete structures at the source and to obtain more durable structures. Various strategies have been applied in the development of self-healing concrete materials and work on new strategies continues at full speed. Increasing the autogenous healing efficiency with various fiber materials [17], [19], increasing the autogenous healing capacity by using superabsorbent polymers [18], [20], repairing cracks with the help of vascular systems formed in concrete or polymers integrated in capsules [22], and repairing cracks biogenically with the help of microorganisms integrated into concrete with different methods can be listed among the strategies studied [23]–[26]. Among these developed concretes, the results obtained with microbial self-healing bio-concrete are very promising. Self-healing for cracks can be investigated by seperating into two groups: Autogeneous healing and autonomous healing.

2.1.1. Autogenous Healing of the Cracks

Concrete is not a best solution for steel because cracking is inevitable. Concerning the protection of steel reinforcement against chemical attacks, however, concrete does have inherent mechanisms which to some degree close the cracks. The process is called "autogeneous healing," and the researchers are making considerable efforts to understand the behaviour. The autogenous healing of a concrete crack is caused by hydration of unhydrated concrete particles, the cracking of the impurity transported through or the sedimentation of fractured parts, the swelling of the crush walls with the formation of calcium silica hydrogen (C-S-H) and, the formation of $CaCO_3$ mineral products through the carbonation of portlandite [27].

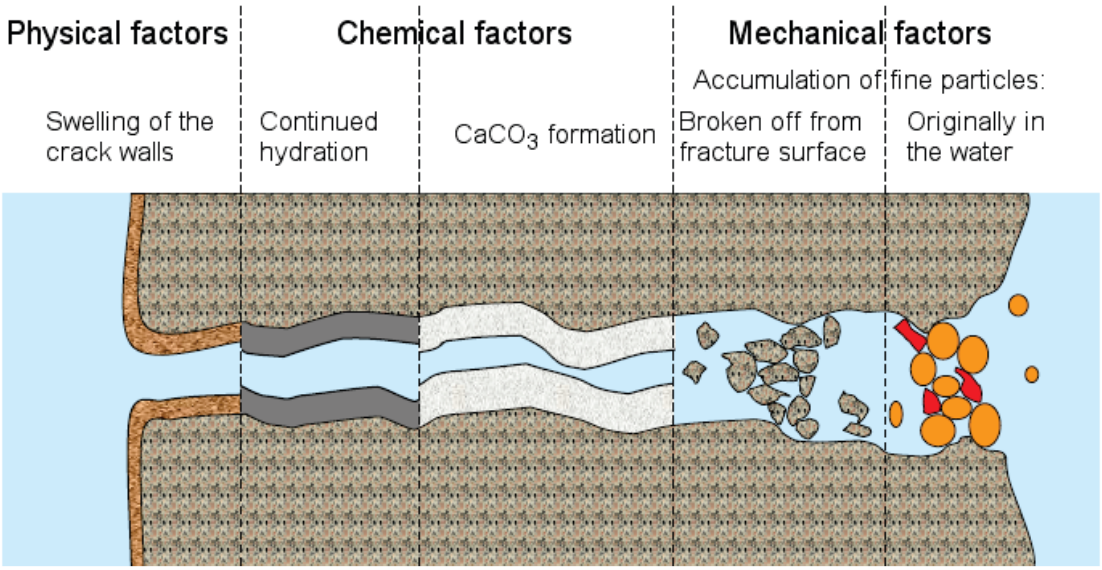

Figure 2. 1.Factors that prompt the autogeneous healing of concrete cracks [27].

Studies exploring the autogenous healing of concrete revealed that the healing capacity of concrete varies between crack widths of 10 μm and 300 μm [26], [28], [29]. The performance of the reported range is varying and difficult to predict. Nevertheless, significant water tightness recovery and partial resistance recovery have been mentioned. The water permeability decrease of cracked concrete was observed by Edvardsen [30] as a consequence of autogenous crack healing, with 80 to 99%. After autogenous healing, Kenneth and Floyd [31]revealed recovery of 25% of tensile strength. These outcomes led to the idea of improving autogenous healing or replacing it with a more stable and consistent phenomenon. Researchers have therefore developed strategies for improving, controlling and predicting the healing of concrete cracks. These contributions can be seen as the practical application of autonomous healing functions.

2.1.2. Autonomous Self-Healing Strategies

The utilization of fracture-induced additives and auto-healing stimulation can be strategic for stable, easy to control and consistent self-healing performance. The strategy based on capsules and vasculars is considered as additives that result from fracture (Figure 2.2).

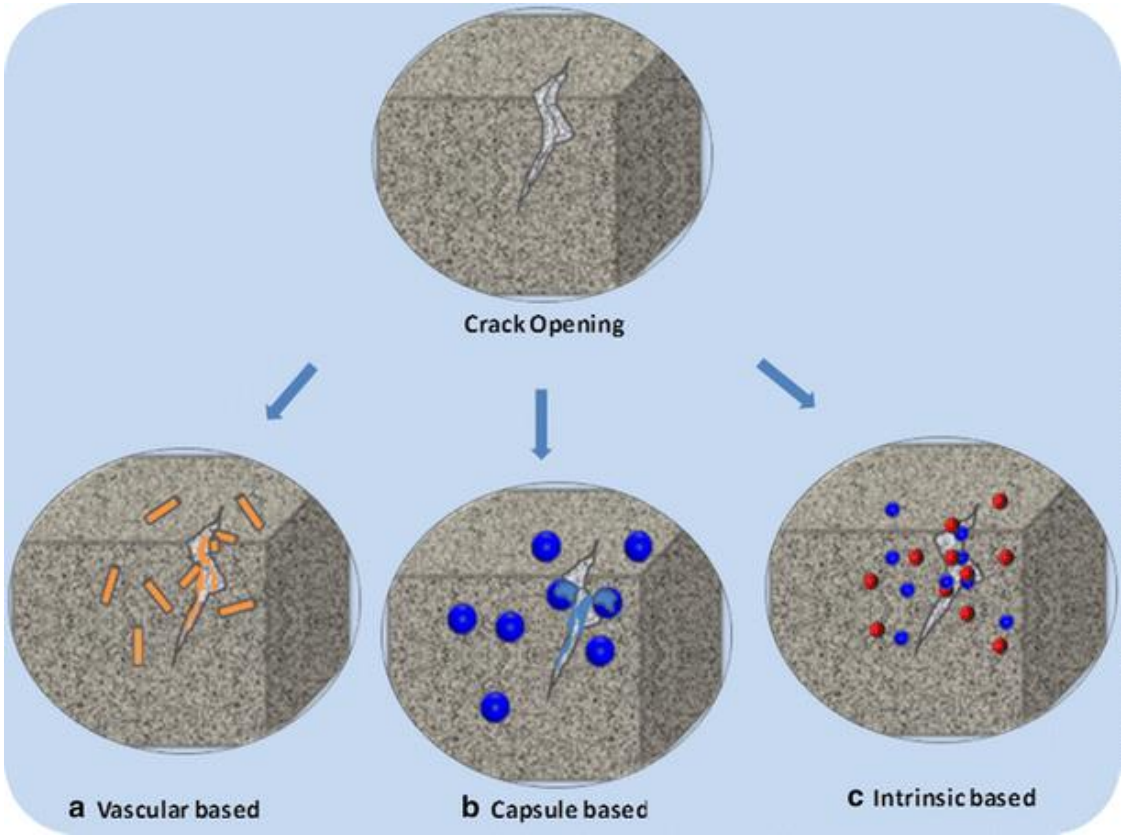

Figure 2. 2. Approaches to designed self-healing cementitious materials. a) Empty canals, filled with a fracture of healing agents, cause damage and leak the healing substance in vascular-based self-healing. b) Healing agent is unveiled from broken capsules by destruction during capsule based self-healing. c) The healing agent has innate self-healing features due to damage or external stimulation in its intrinsic method [32].

Polymer-based healing agents are launched into to the concrete either inner capsules during casting or through vascular systems that after crack has occurred in this method. Regarding autonomous healing of a 400 μm crack width, 48 to 62% strength regain could be accomplished by integrating polyurethane comprising glass tubes in mortar [33]. When epoxy-resin microcapsules have been incorporated into the mortar, bending strength recoveries have been increased up to 1.3 times compared with the reference sample. Compared with autogeneously healed reference sample, the same analysis showed 1.8 times more compressive strength recovery [34]. No optimized system was still accomplished, thus self-healing concrete is being developed through capsule-based healing systems. In comparison to capsule systems, vascular systems have been found to be useful by allowing on-site healing agents where necessary. This strategy was thought

to extend the limits of the healing crack width, but it was observed that the healing agent begins to leak from the crack if the crack is too large [28]. With vascular systems, the width of the healable crack is 0.3 mm [35].

Another way of developing self-healing concrete has been to induce and enhance autogenous healing. For the improvement of self-healing, researchers used shape memory alloys, polymers, natural fibers, bacteria and fibers. It was mainly to limit crack width to measures where autogenous healing is the most efficient method that use fibers or shape memory alloys. Li et al.[11] have presented fiber aided engineered cement composites (ECC). The ECC cracks at several points, resulting in many small-scale micro cracks instead of a single larger crack, allowing the crack width to be maintained to 50 μm. The permeability of the cementitious materials was also limited to a certain level throughout healing using this method. It is possible to obtain reproducible and consistent autogenous healing performances [36]. Furthermore, while ECC was used, cracks in the environment could be healed without using a particular water treatment [37]. Introducing shape memory alloys, that stimulate crack closure throughout structure unloading, was also a strategy used to enhance crack healing [38], [39].

The supply of water inside the crack also is method of stimulation for self-healing. Natural or synthetic 3D cross-liable homopolymers or co - polymers with large ability to absorb liquids are superabsorbent polymers. In cementitious materials with a small water/binder ratio, SAPs were incorporated as an inner curing agent to minimize self-dryer shrinkage throughout hardening [40]. In addition, to mitigate the autogenous shrinkage, SAPs can be incorporated into concrete materials to enhance the freeze-thaw resistance [41] and induce self-determination and self-healing [42]. The self-sealing of cracks occurs immediately after finishing the propagation of cracks and the entry of liquid substances due to the swelling-off effects of the SAPs [18], [43], [44]. It is also able to modify the SAPs as particles, but to be synthesized in situ, that after presence of a crack [45].

In addition to autogenously created $CaCO_3$, Jonkers described a method of immobilized bacterial spores into concrete and hence microbial $CaCO_3$ precipitation (MICP) taken place when crack was formed [46]. $CaCO_3$'s further formation enhanced concrete's ability to self-heal [25].

2.2. Microbial Self-healing of Concrete

Microorganisms' known to develop calcium carbonate extracellularly via biological processes is referred to as MICP. Nadson [47], [48] showed the first comprehensive proofs of the activity of $CaCO_3$ precipitation with bacteria. Chemical precipitation of substances begins with aqueous solutions while their ions are saturated, and the substances cannot be further dissolved. Consequently, an increase of CO_3^{2-} throughout the presence of Ca^{2+} results in precipitation of $CaCO_3$. The bacteria play a significant role in MICP during the first transition. The majority of bacteria were found to affect precipitation of $CaCO_3$ if circumstances permit. Specific bacterial activity leads to the formation of CO_2 in aqueous environments and is partly CO_3^{2-} pursued by $CaCO_3$ precipitate, with Ca^{2+} being present. Bacteria, nevertheless, have an impact not only from the production of CO_2.

The existence of Ca^{2+} ion, dissolved inorganic carbon (DIC), alkalinity, and nucleation zones are indicated as the four major driving factors for MICP by Hammes and Verstraete [49]. In three of the above criteria, bacteria actively participate. Including CO_2, some biological processes result to alkalinity production, that changes the carbonate equilibrium further to CO_3^{2-}. Furthermore, bacteria could function as nucleation sites during in the precipitation method through focusing Ca^{2+} ions around the oppositely charged membrane. The main metabolic activities that can stimulate $CaCO_3$ precipitation in the presence of Ca^{2+} are aerobic or anaerobic respiration, sulfate reduction, aerobic or anaerobic oxidation of organic nitrogen compounds, urea hydrolysis, and photosynthesis. In order to obtain an improvement or provide an alternative, MICP has been evaluated in various areas. MICP use has been described for sandstone formation, soil consolidation and, cementitious surface treatments, and generated waste disposal of Ca^{2+} products [50]–[57]. In addition, the use of MICP constitutes an efficient method for self-healing concrete. The ureolysis pathway is perhaps the most notable route in the application-based MICP research, however, the development of microbial self-healing concrete was studied with aerobic metabolism and ureolysis.

2.2.1. MICP Principles

Biomineralization leads to the development of minerals by microorganisms as a result of the reaction of their metabolic pathways with their surroundings. Mineral production has been described by various species of bacteria such as silicate associated bacteria, urea degrading bacteria, sulfate reducing bacteria (SRB), and unicellular cyanobacteria [58]. Bacteria incorporated cementitious material (bioconcrete) can self-repair microcracks formed on it by evaluating the environmental conditions arising from the crack formation of the bacteria contained in it, multiplying in the crack and precipitating calcium carbonate ($CaCO_3$) in the crack during their metabolic activities. Owing to its large integration with cement-based compositions, calcium carbonate is among the most suitable concrete fillers. Calcium oxide hydration generates calcium hydroxide, capable of reacting to carbon dioxide. These interactions lead to calcium carbonate producing as shown by the Eqs. 1 and 2 [59]. Calcium carbonate is among the most simple and effective filler particles for plugs of voids, porosity and concrete cracks because it is abundant in nature and compatible with cemented compositions [60]. The precipitation of calcium carbonate needs adequate calcium and carbonate ions to surpass the solubility coefficient (Kso) by the ion activity product (IAP). The saturation status while the comparing of the IAP with the Kso (Ω) could be described, i.e., when $\Omega > 1$ is excessive and it is probable that the system is oversaturated [61].

$$Ca^{2+} + CO_3^{2-} \longleftrightarrow CaCO_3 \quad (1)$$

$$\Omega = \alpha(Ca^{2+})\alpha(CO_3^{2-}) / K_{so} \text{ with } K_{so\ calcite,\ 25^o} = 4.8 \times 10^{-9} \quad (2)$$

In the concept of bacteria incorparated cementitious system, crack formation provides the necessary environmental conditions for the bacteria in the form of endospores (also used as spores) to wake up and become active. The carbonate ion concentration is linked to the DIC and pH levels of the aquatic environment concerned. The DIC level also varies based on a few environmental factors, including thermal conditions and CO_2 partial pressure [62]. Calcium carbonate ($CaCO_3$) precipitation can take place by two distinct processes, as with other biomineralization mechanisms: biologically monitored or triggered [63]. The microbe facilitates the process, namely nuclear and mineral particle development, to a significant level during biologically monitored mineralisation. Separate from the rest of natural circumstances, the microbe composes mineral deposits in a type distinctive

towards that species. The bacterial output of calcium carbonate is commonly, but nevertheless, considered to be "induced;" the form of mineral generated depends heavily on natural circumstances [64]. Bacterial species, and also abiotic factors (such as constituents of ambient and salt content), thought to lead to calcium carbonate deposition in a lot of formats in a variety of environments [65]. The first of the environmental conditions formed by crack formation is the release of food that can be consumed by bacteria in the environment [66]. The nutrients used in the content of bioconcrete dissolve in the water entering the crack following crack formation and become usable by bacteria [67]. Another environmental condition that occurs with the formation of cracks is the decrease in the pH of the environment. After the crack formation, the water entering the crack dissolves not only the nutrients in the concrete, but also the $Ca(OH)_2$ molecules that provide the alkaline structure of the concrete. The removal of OH^- ions from the concrete as a result of dissolution causes the pH of the environment to decrease to values between 9.5 and 10.5 [68]. These new pH conditions are suitable for bacteria to consume nutrients and perform their metabolic activities [68].

With the increase in the dissolved nutrient concentration and the decrease in the pH of the environment, the inactive endospores (bacteria in the form of spores) in bioconcrete consume the nutrients necessary to restart their vital activities in anabolic reactions and form active bacterial cells. The newly formed active bacterial cells, on the other hand, consume the dissolved food in the environment with both catabolic (to produce the necessary energy for their metabolism) and anabolic (to create active new cells by growing and multiplying) reactions and carry out microbial activity within the concrete crack. As a result of the catabolic reactions that take place at this stage, CO_2 is released. The released CO_2 provides the formation of $CaCO_3$, which provides the closure of the crack. Different metabolic activities that have been extensively studied in the literature and the $CaCO_3$ precipitation reactions that occur as a result of these metabolic activities are shown in Table 2.1.

Table 2. 1.Microbial $CaCO_3$ precipitation metabolisms and reactions commonly reported in the literature.

Metabolic pathway	**Reactions that cause $CaCO_3$ precipitation as a result of microbial activity**	**Type of Reaction**
	$Ca(C_3H_5O_3)_2 + 6O_2 \rightarrow Ca^{2+} + 4CO_2 + 2HCO_3^- + 4H_2O$	Biochemical

Aerobic oxidation of organic carbon	$4CO_2 + 2HCO_3^- + 6Ca(OH)_2 \rightarrow 6CaCO_3 + 6H_2O + 2OH^-$	Chemical
Urea hydrolysis	$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-}$ $CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3$	Biochemical Chemical
Anoxic oxidation of organic carbon (NO_3^- reduction)	$5Ca(HCOO)_2 + 4NO_3^- \rightarrow 2N_2 + 6HCO_3^- + 4CaCO_3 + 2H_2O + Ca^{2+}$	Biochemical
	$6Ca(OH)_2 + 6HCO_3^- \leftrightarrow 6CaCO_3 + 6H_2O + 6OH^-$	Chemical

2.3. MICP Processes Studied for Microbial Self-Healing Concrete

Calcium carbonate mineral production in MICP is a result of interaction between multiple biological waste products, namely (HCO^{3-}) and, calcium ions in the micro – environment [69], [70]. There are different types of microorganism which are studied for self-healing mechanism throughout MICP. Table 2.1 demonstrates some of these studies and their outcomes.

Table 2. 2. MICP performance by different bacteria and mechanisms.

Bacterial strain	**Pathway**	**Study**	**Crack healing outcomes**
Bacillus alkalinitrilicus *Bacillus cohnii*	Aerobic oxidation of organic carbon	[25], [71]	Crack-healing of up to 0.46 mm wide cracks in bioconcrete was obtained. The crack sealing performance of the lightweight mortar that introduced bacteria is enhanced.
Sporosarcina pasteurii	Ureolytic	[46], [72], [73]	No compatibility problem was observed.

	process		
Bacillus lentus			Capillary water absorption of the treated limestone was considerably reduced by strain.
Bacillus sphaericus			Biodegradation led to a decline in water permeability due to crack sealing.
Diaphorobacter nitroreducens, Bacillus sphaericus	Anoxic oxidation of organic carbon	[66]	Calcite precipitation developed adequate crack closing up to 0.5 mm crack width.
Bacillus subtilis	Non-ureolytic	[74]	Within 28 days, cracks width up to 1.2 mm were fully healed.
Bacillus sp. CT5	Unidentified urease producing	[75]	Bacteria added to the cement mix postponed setting time.
Synechococcus PCC8806	Photosynthesis	[76]	The bacteria produced 200 μm-270 μm calcium carbonate coating upon on concrete surface.
Bacillus mucilaginous	Carbonic anhydrase	[77]	Healing was observed in cracks up to 0.5 mm.

Different bacteria have already been screened as well as a few useful strain for microbial

self-healing concrete improvement has been outlined. *Sporosarcina pasteurii* has been MICP's most extensively investigated bacterial in latest years, followed by *Bacillus subtilis, Bacillus sphaericus, Bacillus mucilaginous, and Bacillus megaterium* species. Additional bacteria, including such cyanobacteria, sulfate and nitrate reducing bacteria have been also evaluated for the possible MICP. In addition, researchers have been tried to isolate bacteria from different environments to get new bacterial strains capable of causing MICP.

Microbial concrete and mortar have been produced using a wide range of bacteria. Because of distinctions in enzymatic mechanism, size, and reaction progression to trigger $CaCO_3$ precipitates, the type of bacteria had a significant impact on the performance of the concluding MICP products. Mostly, microbial cements or mortars show a resilience and durability that is same or greater than normal concretes and mortar. More self healing potential has been achieved with bacterial concrete or mortar, which can heal 0.5 mm–1.0 mm large cracks [77]. Because of the alkaline environment of the cementitious materials, it is preferable to protect the microorganisms in a carrier by encapsulation or immobilization before using them to the cementitious materials to enhance bacterial spore intensity and service life[78], [79]. Studies have previously suggested various types of bacterial protective methods and equipment. Diatomaceous earth (DE) was used to immobilize bacteria spores, however the smaller grain sizes of DE reduce bacteria loading efficiency [80]. In the bacterial protection and healing of cracks with widths of up to 0.46 mm, Jonkers and Wiktor (36] utilised light weight aggregate (LWA) [81]. In addition, polyurethane and silica gel were used to immobilize bacteria and nutrients and experiments indicated a greater strength recovery (60%) for cracked mortar samples cured by polyurethane immobilised bacteria than for silica gel immobilised bacteria, which demonstrated a strength recovery of just 5%. [82]. Another study investigated the effectiveness of expanded perlite (EP) as a novel bacteria protection for evaluating crack-healing in concrete through immobilization of *Bacillus cohnii*. The results of the experiments revealed that samples introduced with EP-immobilized bacteria demonstrated effective crack-healing by up to 0.79 mm healed crack width in 28-days [83]. Additionally, in another study, a patented polymer-condensation microencapsulation process was used to encapsulate bacterial spores. The results revealed that healing rate was greater in the samples with bio-microcapsules (48-80%) than in samples without bacteria (18-50%) [84]. The other study examined at self-healing using

hydrogel-encapsulated bacterial spores (bio-hydrogels). For those cracks less than 0,3 mm, the healing rates for samples with bio-hydrogels were between 70% and 100% [85].

The first tests to develop self-repairing bioconcrete by microbial means based on biogenic $CaCO_3$ precipitation were carried out using *Bacillus alkalinitrilicus* bacteria, which aerobically oxidizes calcium lactate [25], [46]. In this study, it was stated that *Bacillus alkalinitrilicus* and calcium lactate were placed in the pores on the expanded clay using vacuum impregnation and loaded expanded clay particles contained 6.0% by weight of calcium lactate and 1.7×10^5 bacterial spores/g particles on it. The resulting expanded clay particles were added to the mortar mix as a microbial repair agent (76% by weight of cement) to replace 24% of the fine aggregates, and mortar samples containing 5.0×10^{10} bacterial spores/m^3 of mortar were obtained. In the results obtained from the tests performed on these samples, it was reported that the 0.46 mm wide cracks formed on the mortar samples healed spontaneously when the mortar samples were cured in water for 100 days [25]. In another study using aerobic heterotrophic bacteria, *Bacillus halmapalus* bacteria, which are active at lower temperatures (8°C) and high salinity (30 g/L NaCl), were immobilized in calcium alginate grains to obtain biomortar samples. When the samples obtained were tested for 400 μm and 600 μm cracks, it was reported that 400 μm and 600 μm cracks in bacterial samples were closed after 56 days of seawater curing. As a result of the closure of the cracks, it was reported that the water permeability of 400 μm and 600 μm cracks decreased by 95% and 93%, respectively [86]. In another study, it was reported that the flow values of the mortar samples with similar content were 30% higher and the porosity almost 3 times higher than the reference sample. In the compressive strength tests performed with these samples on the 3rd, 7th and 28th days, the strength values obtained with the bacterial samples were 68%, 30% and 35% higher than the reference sample since some of the fine aggregates were replaced by expanded clay containing bacteria reported to be low [71]. However, in the same study, when the bacterial samples were compared with the control samples in which the fine aggregates were replaced with bacteria-free expanded clay, it was stated that there was a 60% decrease in the compressive strength values only on the 3rd day, and the compressive strength values on the 7th and 28th days were similar. It has been reported that the effect of aerobic bacteria placed in expanded clay on the early age strength is negative, but there is no negative effect on the strengths obtained on the 7th and 28th days. In self-healing tests with cracked mortar samples of the same content, it was stated that a 350 μm crack

self-closed within 28 days in samples with bacteria cured both in completely submerged conditions and in wet/dry cycles. It was reported that samples with bacteria regained their waterproofness by 69% and 91% at 28 and 56 days, respectively [71]. Studies for large-scale testing of isolated aerobic heterotrophs are ongoing and no results have yet been published [87].

The only disadvantage of aerobic heterotrophs in the development of self-healing concrete by microbial means is that metabolic reactions do not contribute to the alkalinity required for calcium carbonate precipitation and the precipitation reaction is completely dependent on the alkalinity in the concrete (Table 2.1). Since $Ca(OH)_2$ in concrete is consumed during crack repair, crack repair may indirectly accelerate the carbonation of concrete.

After the first tests with aerobic heterotrophs, urolytic bacteria that perform urea hydrolysis metabolic pathway and urea hydrolysis, which provide more $CaCO_3$ precipitation in a shorter time compared to aerobic oxidation of organic carbon and do not need external alkalinity for this process (Table 2.1), have been started to be tested [23], [26], [80]. In the first study using only urolytic bacteria, they obtained a self-healing biomortar of 150-170 μm cracks by using the immobilized effects of *Bacillus sphaericus* in diatomaceous earth [80]. After that, when the same bacterial species were immobilized in microcapsules, a significant increase in the crack healing performance of the developed biomortars was observed. It has been reported that biomortar samples containing 3.0% and 5.0% by weight of cement microcapsules with bacteria can repair 400 μm wide cracks within 56 days [26]. In another study, *Bacillus sphaericus* spores were individually immobilized with zeolite, metakaolin and air entrainer containing 0.1 g spore/g material, and the setting and compression strengths of microbial self-healing biomortars obtained using these materials were tested [88]. The setting start times of the bio-mortars containing 5.0% bacterial zeolite, 5.0% bacterial metakaolin and 1.0% bacterial air entrainer by weight of cement were determined as 210 minutes, 300 minutes and 270 minutes, respectively, and setting end times were 520 minutes, respectively , was determined as 540 minutes and 450 minutes [88]. Among these mixtures, it was reported that the compressive strengths of the mortar mixtures containing bacterial metakaolin and bacterial air entrainer were 30% lower than the reference sample at the 7th and 28th days. It was stated that the mortar containing zeolite with bacteria had approximately 10%

lower compressive strength compared to the reference sample on the 7th and 28th days [88]. In another study, *Bacillus sphaericus* spores were added directly (without immobilization) at the rate of 0.5% and 1.0% by cement weight, and it was reported that the compressive strength of the obtained biomortar sample was 60% lower than that of the reference mortar sample [89]. When the unimmobilized bacterial spore dose was increased to 3.0% and 5.0% by weight of cement, it was stated that no measurable compressive strength could be obtained even after 56 days [89]. On the other hand, although the 2nd day compressive strength of the biomortars prepared using 0.5% and 1.0% by weight of cement, *Bacillus sphaericus* spores immobilized with diatomaceous earth, are 38% and 50% less, respectively, compared to the reference mortar sample; it has been reported that the compressive strength at the end of 56 days is only 10% lower than the reference sample [89]. It was stated that the biomortar sample prepared by using 3.0% cement weight and *Bacillus sphaericus* spores immobilized with diatomaceous earth showed 55% lower compressive strength compared to the reference sample at the end of 56 days. Another widely studied urolytic bacteria species for the urea hydrolysis metabolic pathway is *Bacillus pasteurii*. In a recent study, it was reported that mortar samples containing 1.4×10^{13} bacterial spores/m^3 of mortar were able to spontaneously repair cracks up to 300 μm wide in 28 days [90].

The application of axenic cultures was favored in the first microbial self-healing investigations because these studies primarily consider evidence of healing ideas through the study of different metabolic pathways. The observed type of microbial self-healing cementitious materials, on the other hand, can cost up to 2400 EUR/m^3 [91]. The aseptic manufacturing process of axenic cultures and effective carriers (such as the abovementioned microencapsulation etc.) for the introduction of bacteria into concrete account for the high prices [91]. Non-axenic biogranules can be introduced as a promising generation bacterial healing agent as a cost-effective option to axenic cultures.

A non-axenic urolytic culture was developed for use in self-healing cementitious composites developed using urolytic bacteria [29]. This mixed culture, named "Cyclic Enriched Urolytic Powder" (CERUP), which is obtained by culturing different urolytic bacteria together, eliminates the need for extra protective material, and when used at a rate of 1.0% and 2.0% by cement weight, 2-day, 7-day; it has been reported that there is no negative effect on the 28-day and 52-day compressive strength values [29]. It has been

stated that biomortars obtained by using 2.0% CERUP by weight of cement can spontaneously repair crack widths up to 400 μm within 28 days. However, there is no information about the effects of using different doses of CERUP on strength, setting and self-healing capacity.

In a recent study, the 3.5 m^3 ceiling slab of one of the control points of the wastewater collection line was made with concrete prepared using 1.0% CERUP by weight of cement. Concrete with dimensions of 100 mm × 100 mm × 400 mm poured using the same mixture was cracked in the laboratory and its self-healing performance was monitored for 6 weeks [92]. It has been reported that the best performance is obtained in wet/dry cycles, and that bioconcrete can self-heal cracks up to 400 μm when cured with wet/dry cycles for 6 weeks, and can heal cracks up to 200 μm in 6 weeks when curing submerged in water. It was especially emphasized that the self-healing performance could not be examined since there is no crack in the concrete in the application area yet. Another recent study introduced the use of anaerobic granular sludge as another alternative bioconcrete for the mitigation of sewer corrosion. The corrosion rates of 1% and 2% (of the cement weight) bioconcrete were approximately 17.2% and 42.8% lower than that of the control concrete [93].

2.3.1. Cementitious Composites that Self-heal via Nitrate Reduction

In both of the above-mentioned metabolisms (aerobic oxidation, urea hydrolysis) bacteria are directly or indirectly dependent on dissolved oxygen, and because the by-products (NH_4^+ and NH_3) formed as a result of urea hydrolysis are harmful to the binders in concrete and living organisms in aquatic environments [94], an alternative metabolic route necessity has emerged. In this context, it has been stated that nitrate-reducing bacteria (which can oxidize organic carbon to CO_2 under anoxic conditions by using nitrate instead of oxygen) can be a more environmentally friendly and more efficient alternative for biomineralization in environments such as cracks where oxygen is limited [66], [95]. Since the nitrate reduction metabolic pathway also produces alkalinity, it requires less concrete alkalinity compared to aerobic oxidation of organic carbon (Table 2.1). The operability of the nitrate reduction (denitrification) metabolic pathway was demonstrated using nitrate-reducing axenic bacteria, *Diaphorobacter nitroreducens* and *Pseudomonas aeruginosa* [95]. In this study, in which expanded clay and granular

activated carbon were used to protect the bacteria in the mortar, each bacterial species was loaded on the protective porous carriers at an average rate of 0.1 g/g using vacuum impregnation method. Mortar samples containing bacteria at the rate of 0.5% by weight of cement were prepared by using granular activated carbon with bacteria and expanded clay particles with bacteria. It has been reported that the obtained mortar samples can self-heal cracks up to 370±20 μm wide in 28 days and self-repair cracks up to 480±16 μm wide in 56 days [95]. It has been stated that up to 85% of waterproofing can be recovered in healed cracks. In addition, since the activity of bacteria is independent of dissolved oxygen, it has been reported that, unlike other metabolic pathways, bacteria are active in the depths of the cracks and heal in the form of layers [95]. Since the protective porous carriers used in the study were used in addition to fine aggregates, it was stated that there was no loss of strength. In another study, the compressive strengths of the mortar samples containing *Diaphorobacter nitroreducens*, which were loaded separately on diatomic soil, expanded clay and granular activated carbon at a rate of 0.1 g/g, were compared with the reference mortar sample and the mortar sample containing bacterial diatomaceous earth was compared at the 7th and 28th grades. It has been reported that the compressive strengths obtained per day are the same as the reference sample, and the compressive strengths of the mortar samples containing expanded clay with bacteria and granular activated carbon with bacteria are 15% and 20% better, respectively, than the reference mortar sample, regardless of the day [88].

Non-axenic cultures could be used in cementitious materials in the form of granular bacterial cultures [66]. The methodical placing of bacterial cultures in a compact form is one of the major benefits of granulated bacterial cultures [96]. Denitrifiers, polyphosphate collecting bacteria, nitrifiers, and aerobic heterotrophs can live side by side in granules [96]. Furthermore, for the preservation of the core bacteria, layered structure and the compact shape of granular biomass are beneficial [97]. Researches have shown that, where necessary, granular bacteria can be dried and stored in order to reactivate later [98]. Ersan et al. [66] developed non-axenic nitrate-reducing biogranules for use in self-healing cementitious composites developed using nitrate-reducing bacteria. The biogranules obtained by the production of different nitrate-reducing bacteria in the biogranulation reactor are called “Activated Compact Denitrfying Core” (ACDC). The bacterial content of ACDC granules was stated as 0.7g/g, and it was reported that these granules eliminate the need for extra protective material and do not have a negative effect on the 7 and 28

day compression strength when used at a rate of 0.70% by cement weight [88]. In addition, at the same dose, it was stated that the setting start time was 180 minutes and the set end time was 310 minutes [88]. The use of Activated Compact Denitrifying Core (ACDC) biogranules in accordance with certain concrete admixtures enhanced the self-healing ability of the mortar samples. In addition to being effective for early age cracks, microbial self-healing using a culture of ACDC closes cracks occurring in aged samples. Microbial induced $CaCO_3$ minerals have similar mechanical properties to the autogenously formed $CaCO_3$ minerals when compared through micro indentation [66]. Additionaly, biomortar samples containing 0.70% ACDC granules by weight of cement can self-repair cracks up to approximately 0.50 mm wide within 28 days, the waterproofing of healed 400 μm cracks is 83% better than autogenous healing, and the cracks can be repaired by itself. It has been reported that the calcium carbonate layer that closes can reach up to 1 cm thick in places [66], [99]. However, there is no information on how these biogranules affect the setting time, mechanical properties and self-healing performance when used in different doses in the studies conducted so far.

Some bacterial species that have been tested for bioconcrete development and their metabolisms are given in Table 2.2. At the last point, it has been reported that mixed cultures such as non-axenic CERUP, ACDC are more practical in terms of use in large scales and more suitable in terms of production costs compared to axenic cultures [29], [100]. Considering the adverse effects of the different metabolic pathways investigated for the development of self-healing concrete by microbial means on the concrete matrix and aquatic environments (Table 2.1), it can be said that nitrate-reducing biogranules, the by-product of which is inert nitrogen gas, are slightly more advantageous than the other microorganisms and microbial cultures examined.

2.4. Up to Date Research Needs for MICP

Priority has been given to the functionality of different metabolic pathways and the proving of the concept of "bioconcrete that self-repairs by microbial means" in the studies carried out to date. Variables for the mix design of self-healing bioconcrete by microbial means are mostly ignored. In the studies, almost all of which were carried out at laboratory scale, mortar mixtures were used to represent concrete samples, and analyzes were carried out to determine whether the designed system was functioning by putting

both nutrients and bacteria in the mortar more than necessary. One of the materials used in the production of self-repairing bioconcrete by microbial means, at least as important as bacteria, is the nutrients added to the mixture and consumed by the bacteria during their proliferation and energy production activities after crack formation. At the last point, calcium formate and calcium nitrate are used as nutrients in self-healing cementitious composites obtained by using biogranules (ACDC) by microbial means [66], [99], [101]. As seen in Equation 3, these chemical additives directly affect the precipitated $CaCO_3$ amount. In a recent study on the optimization of nutrient content, in cementitious composites that repair itself microbially through nitrate reduction, considering the workability, mechanical properties and the amount of nutrients available to bacteria in case of cracks, the optimum amount of nutrients to be used in the mixture is 5% calcium formate and 2% by weight of cement. determined as calcium nitrate [67].

$$5Ca(HCOO)_2 + 2Ca(NO_3)_2 + 3Ca(OH)_2 \rightarrow 2N_2 + 10CaCO_3 + 8H_2O \quad (3)$$

In addition to nutrient optimization studies, the optimization of bacteria amount is also of great importance. When the studies in the literature are examined, it has been reported that different bacterial species and concentrations have different effects on strength and setting [71], [88], [89], [102]–[104]. In addition, the minimum amount of bacteria required in the environment in terms of $CaCO_3$ precipitation and the effect of increasing bacteria amount on $CaCO_3$ precipitation efficiency have been stated in previous studies [49], [105], [106]. It is thought that the amount of precipitated $CaCO_3$ and the precipitation efficiency will also directly affect the healing of concrete cracks. However, there has not been a comprehensive study on the optimization of the bacterial amount and the minimum amount of bacteria required to obtain self-repairing bioconcrete by microbial means.

2.5. The Aim and Scope of the Thesis

Bacteria used in the content constitute a large part of the cost of self-healing bioconcrete [91]. Despite its great importance, unfortunately, the amount of bacteria used in self-healing cementitious composites, which have been developed up to now and have achieved positive results, has not been determined by considering any reference. The minimum amount of bacteria that bioconcrete should contain for self-repair has not been

specified, and no study has been conducted on the changes in healing capacity and speed depending on the amount of bacteria.

Conducting a study covering these requirements is of great importance in terms of clarifying the mixture composition of self-healing bioconcrete according to certain parameters and minimizing unnecessary costs. From the point of view of Turkey, the number of studies on self-healing cementitious composites by microbial means is negligible. Bioconcrete technology that repairs itself by microbial means has been heard recently and started to be researched with limited knowledge. The researches mostly repeat the existing literature and make limited contribution to the development of technology both in the world and in Turkey.

Considering all abovementioned requirements, the scope of this thesis has been created on concrete and cementitious composites that are self-healing with biogenic nitrate reduction, which is described as an environmentally friendly process at the last point of the literature. In this context, bacteria were tested in the form of nitrate-reducing biogranules. In this completed thesis study, the effect of the amount of bacteria used in the microbial self-healing cementitious composites on the setting time, compressive strength and self-healing capacity of the mortar was investigated. In addition, the minimum amount of bacteria in the form of biogranules required to obtain a self-healing mortar mixture by microbial means was determined. By determining the required minimum amount of biogranules, the new cost of microbial self-healing bioconcrete was determined and compared with the costs reported in previous studies. It is thought that the information obtained will contribute to repairing wider cracks in bioconcrete that heals itself by microbial means, providing surface closures as well as volumetric improvements and reducing the cost of technology.

3. MATERIALS AND METHODOLOGY

The production of nitrate reducing biogranules, preparation of bacterial and non-bacterial mortar samples, tests and analyses on fresh and hardened mortar samples are explained in detail in this section.

3.1. Production of Biogranules Containing Nitrate-Reducing Bacteria

The vaccine sludge required for the production of biogranules was taken from the advanced biological waste water treatment plant of Kayseri Sugar Factory. The amount of water has been reduced as much as possible by settling the collected vaccine sludge. The amounts of suspended solids (TSS) and volatile suspended solids (VSS) of the resulting dense sludge, are given in Table 3.1.

Table 3. 1. VSS and TSS content of concentrated grafting sludge.

Parameter[1]	**Amount**
TSS (mg/L)	24360
VSS (mg/L)	21924
pH	7.7

[1]VSS: Volatile suspended solids; TSS: Total suspended solids

3 bottles of 1 L each were filled with concentrated vaccine sludge, and a total of 3 L graft sludge was pasteurized to purify it from possible vegetative pathogens. In the pasteurization process, glass bottles filled with mud were heated up to 80 ºC in a water bath and kept at this temperature for 30 minutes, then cooled in an ice-filled container. Since the microorganisms that could not form spores in the graft sludge died at the end of pasteurization, a lower concentration of nutrients was given in the first days of operation in order for the system to get used to the new operating conditions and for the growth of resistant microorganisms remaining in the sludge.

However, in the thesis, dried forms of biogranules, which were previously prepared by precipitation and various processes, were used. Biogranules are produced by feeding from their dried form. Afterwards, the dry biogranules were placed in a sequential batch reactor (SBR) (Figure 3.1) with an inner diameter of 124 mm and an effective height of 60 cm,

and the reactor was operated with a 50% volume change. Feeding and discharging of the nutrients used were carried out in the same period, and a 50% volume change was realized in this period. Fresh food was fed by a peristaltic pump from the bottom of the reactor, while the consumed nutrient solution was collected from the top of the reactor. The reactor was operated for a total of 240 days.

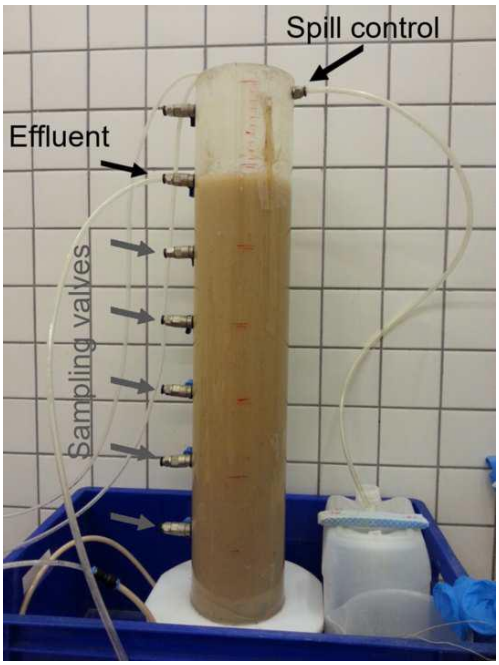

Figure 3. 1. Biogranule production reactor with a total effective operating volume of 7.24 L and operated at a 50% volume change rate.

The operating method and parameters applied in the SBR for the granulation of bacteria are compatible with the biogranule production applied in the previous studies of the thesis co-advisor [66], [97]. In order to ensure the selection of biogranules that can be integrated into the concrete, the pH of the nutrient solution was kept at 10 during the operation.

Since the biogranules produced in the reactor will be used in the mortar samples that do not contain vitamins and micronutrients, the reactor was fed with a minimal nutrient solution (nutrient free of vitamins and micronutrients). The nutrient mixture used after the total COD and TN concentrations were fixed is given in Table 3.2 [107].

Table 3. 2. The content of the nutrient solution used in the production of biogranules.

Nutrients	Concentration (mg/L)
NaHCOO	3239
$NaNO_3$	331
$Ca(NO_3)_2$	267
$MgSO_4.7H_2O$	89
KH_2PO_4	13

3.1.1. Monitored Parameters for Biogranulation

The granulation process of the bacteria was continuously monitored by sludge volume indices (SVI) of 30 minutes and 5 minutes. The SVI_{30} / SVI_5 ratio used in biogranulation studies in the literature was used to determine the percentage of granules in the reactor [108]. In addition, in order to understand whether the operated reactor content is granular or not, the 50 ml / g limit SVI_{30} value specified in previous studies was also followed [109]

In the literature, sizes up to 200 μm are classified as flock, and pellets larger than 200 μm are classified as granules [110]. Therefore, the percentage of granules in the reactor was evaluated by performing sieve analysis into the 750 mL mixture taken when the reactor was fully mixed. Steel screens of 0.210 mm, 0.450 mm, 0.850 mm, 1.00 mm and 2.00 mm were used in the sieve analysis. Granule percentage was calculated using the formula in Equation 4.

$$Granule\ percentage = \frac{m_{d>0,210mm}}{m_T} \times 100 \quad (4)$$

md> 0,210mm: Dry weight of bacterial balls larger than 0,210 mm,
mT: the dry weight of the total bacterial culture in the sieved solution.

3.1.2. Quality Control of Produced Biogranules

The presence of the desired denitrification bacteria in the center of the biogranules was monitored by kinetic measurements (sampling every 15 minutes) performed during the aerobic period during the SBR operation over total nitrogen removal and COD removal.

Nitrate and COD removal in the anoxic period was performed to determine the presence of nitrate reducing bacteria, and the kinetic monitoring of the nitrate removal and total nitrogen amount in the aerobic period was performed to determine whether the nitrate reducing bacteria were at the center of the biogranules.

By performing Fourier transform infrared (FTIR) analysis on the biogranules, it was determined whether the desired $CaCO_3$ and $Ca_3(PO_4)_2$ minerals were present in the outer part of the granules. In addition, the granules were imaged under the scanning electron microscope (SEM) combined with energy dispersive X-ray spectroscopy (EDS) and the distribution of the minerals in the biogranules within the granule was determined by element mapping.

The 1- and 24-hour water absorption amounts of the produced biogranules were tested. 1 g of dried biogranule was taken and kept in tap water for 1 hour (Figure 3.2-c). Afterwards, the filtration device was operated and all the water was passed through the filter (Figure 3.2-b). The water absorption amount was calculated using the weight of the water absorbed granules remaining on the filter. The same procedure was repeated, keeping 1 g of dry granules under water for 24 hours (Figure 3.2-a).

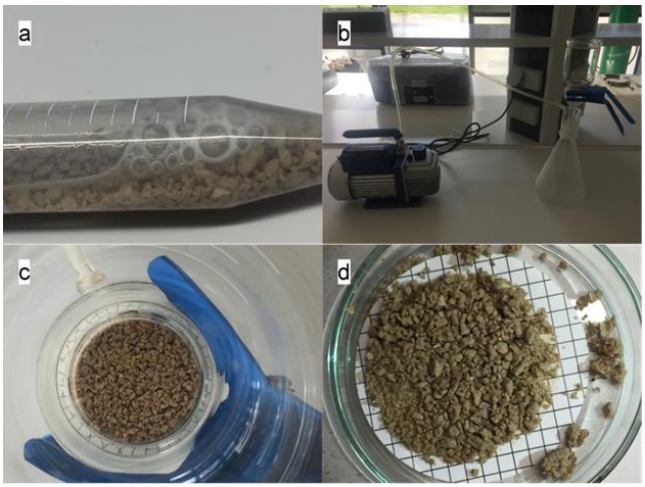

Figure 3. 2. Vacuum filtration test setup to determine water absorption of dry biogranules; (a) dry biogranules soaked in water for 1 h and 24 h; (b) vacuum filtration setup; (c) filtration of non-absorbed water; (d) surface saturated dry biogranules.

3.2. Methodology

This section of the thesis explains in detail the mixture development, preparation and experiments used in the study.

Biogranule samples taken from the reactor were generally analyzed following sampling. In cases where the analyzes could not be performed immediately, the biogranule samples were stored in the refrigerator at +4°C. SVI measurements in the reactor were carried out in accordance with the protocol given in standard methods [111]. TSS and VSS analyzes on biogranule samples taken from the reactor were also performed using a 105 °C drying oven and 650 °C muffle furnace according to the procedure described in standard methods [111].

The dried biogranules were pulverized with the help of a pestle and a portion of the obtained powder was taken (< 5mg) and the FTIR spectrum was obtained. The FTIR spectra presented in the thesis were obtained from 32 scanning results in the range of 4000–500 cm^{-1}, with a resolution of 4 cm^{-1}.

Some of the samples taken randomly from the dried biogranules were divided into two parts and some were viewed as a whole under scanning electron microscopy (SEM) combined with energy dispersive X-ray spectroscopy (EDS). Biogranules were coated with carbon before imaging. Micrographs of the coated biogranules were obtained using a secondary electron detector under 15 kV voltage. In addition, Ca, P and O elements were mapped in biogranules and bisected biogranules with the help of EDS, and the distribution of minerals and bacteria in biogranules was determined.

3.2.1. Preparation of Mortar Samples and Determination of Their Self-healing Capacities

Since the results obtained from mortar samples can be used to represent the results obtained from concrete, it is more advantageous to use mortar samples in laboratory-scale tests of new technologies. For this reason, the mortar samples would be used to represent the concrete samples. Therefore, the tests in this thesis study were made on the mortar and the mortar samples were prepared in accordance with the EN 196-1 standard with a

sand: cement: water ratio of 3.0: 1.0: 0.5. DIN EN 196-1 sand (1350 g), CEM I 42,5R cement (450 g) and tap water (225 g) were used as basic mortar components. Reference samples were prepared with this mixture. Apart from the reference sample, nutrient-only abiotic control samples (referred to throughout the text as "control sample") were prepared and in addition to the basic mortar components, 5.00% calcium formate (22.50 g) and 2.00% calcium nitrate (9.00 g) was used.

The main differences of biomortar mixes from normal mortar mixes are the bacteria in its content, bacteria carriers, if any, and nutrients required for the activity of bacteria. In this study, the amount of nutrients used in biomass was fixed as 5.00% calcium formate and 2.00% calcium nitrate by weight of cement. These values were determined on the basis of the optimization study [67] conducted by co-advisor's previous studies by evaluating the amount of nutrients available for bacteria after crack formation in cement composites with different nutrient content, which are self-repairing by microbial means.

While preparing the samples of bio-mortar, in addition to the basic mortar components and nutrients, different doses of bacteria (varying between 0.25% and 3.00% by weight of cement) were added in the form of biogranules produced in the biogranule reactor and passed quality control tests, and the increase in the amount of biogranules. The effects on 3-, 7-, 28- and 56-days compressive strength were investigated. Thus, while developing cementitious composites that repair themselves by microbial means, the maximum amount of bacteria in the form of biogranules that can be used without negatively affecting fresh and hardened mortar behavior was determined in the mixture. When determining the effect of the bacteria dose, the behavior of the abiotic control sample that did not contain bacteria but only contained nutrients rather than the reference sample was taken into account. Starting from the maximum amount of bacteria determined according to these criteria (2.50% by weight of cement), the amount of bacteria in the form of biogranules is reduced in steps of 0.25% by weight of cement, and all mixtures between 2.50% and 0.25% have self-repair capacity and self-repair speed. It has been evaluated in terms. Finally, the minimum amount of bacteria required to obtain a cementitious composite that repairs itself by microbial means was determined by comparing the self-healing capacity of the mortars prepared using the doses of 0.05% and 0.10% by weight of the cement compared to the control and reference sample. While examining the mortar

properties and self-healing capacities, the prepared mortar mixtures and their contents are given in Table 3.3.

The particle sizes of the biogranules used in the content of bio-mortar samples vary between 0.45 and 2.00 mm. The particle size distribution of the biogranules was kept the same in all the biomass samples tested in this study. Accordingly, 50% by weight of the total amount of biogranules used in each bio-mortar mixture is from biogranules with a size of 1.00-2.00 mm, 35% by weight is from 0.85-1.00 mm in size and 15% by weight is 0.45. It consists of –0.85 mm sized biogranules. The amount of biogranules of different sizes added to different bio-mortar mixtures is given in Table 3.3.

While determining the minimum bacteria dose, values below 0.05% by weight of cement could not be tested within the scope of this study. Because, with the particle size distribution tested within the scope of this thesis, biogranules with dimensions between 0.45-2.00 mm (0.45-0.85 mm by 15%, 0.85-1.00 mm at 35%, 1.00–2.00 mm by 50%). It is not possible to obtain a homogeneous mixture when is added in doses less than 0.05% by weight of cement. Since this situation will significantly reduce the possibility of possible cracks to coincide with biogranules, it was predicted that healthy results could not be obtained and lower doses were not tested.

Table 3. 3. The mortar mixes and their contents prepared in different test sets.

Research question	**Mortar mixtures[1]**	**Biogranule[2] amount (g)**			**CF[3] (g)**	**CN (g)**
		0.45 – 0.85 mm	**0.85 – 1.00 mm**	**1.00 – 2.00 mm**		
Maximum amount of bacteria that can be used	Reference	-	-	-	-	-
	Control	-	-	-	22.50	9.00
	Bio-0.25%	0.24	0.56	0.81	22.50	9.00
	Bio-0.50%	0.48	1.12	1.61	22.50	9.00
	Bio-0.75%	0.72	1.69	2.41	22.50	9.00
	Bio-1.00%	0.97	2.25	3.21	22.50	9.00
	Bio-1.50%	1.45	3.37	4.82	22.50	9.00
	Bio-2.00%	1.93	4.50	6.43	22.50	9.00
	Bio-2.50%	2.41	5.62	8.04	22.50	9.00
	Bio-3.00%	2.89	6.75	9.64	22.50	9.00

	Reference	-	-	-	-	-
	Control	-	-	-	22.50	9.00
	Bio-0.25%	0.24	0.56	0.81	22.50	9.00
	Bio-0.50%	0.48	1.12	1.61	22.50	9.00
The effect of bacteria dose on self-healing capacity	Bio-0.75%	0.72	1.69	2.41	22.50	9.00
	Bio-1.00%	0.97	2.25	3.21	22.50	9.00
	Bio-1.25%	1.21	2.81	4.02	22.50	9.00
	Bio-1.50%	1.45	3.37	4.82	22.50	9.00
	Bio-1.75%	1.70	3.94	5.61	22.50	9.00
	Bio-2.00%	1.93	4.50	6.43	22.50	9.00
	Bio-2.25%	2.17	5.06	7.23	22.50	9.00
	Bio-2.50%	2.41	5.62	8.04	22.50	9.00
Minimum amount of bacteria required for microbial healing	Bio-0.05%	0.05	0.11	0.16	22.50	9.00
	Bio-0.10%	0.10	0.23	0.32	22.50	9.00

[1] Sand: cement: water quantities 1350 g: 450 g: 225 g are kept constant in all mixtures, and the percentages given in bio-mortars show the amount of bacteria by weight of cement.

[2] Biogranules contain 70% bacteria and 30% mineral by weight.

[3] CF: Calcium formate. CN: Calcium nitrate.

3.2.2. Tests on Fresh Mortar Samples

Setting experiments were performed according to ASTMC807 standard using automatic vicat equipment (Matest E0440N Vicatronic, Italy). The results obtained were evaluated considering the minimum setting starting time of 60 ± 12 minutes and the minimum setting termination time of 90 ± 20 minutes given in ASTM C191-04 and NBN EN 1008 standards. The 12-minute and 20-minute standard deviations given in the limit values are the standard deviation values given for the tests carried out by a single operator in the ASTM C191-04 standard.

Flow table tests on fresh mortar samples were made according to the NBN EN 1015-3 standard. In the NBN EN 1015-3 standard, it is stated that the flow value of the tested sample must differ by at least 10% from the flow value of the reference sample in order for the flow properties of a mortar mixture to be different from the reference mortar

mixture. While evaluating the results of the flow tests performed in this thesis in terms of consistency and workability, the deviation limit of 10% specified in the NBN EN 1015-3 standard was taken into account, and comparisons of bio-mortar were made according to both reference and control samples.

3.2.3. Tests on Cured Mortar Samples

Compressive strength tests were carried out on the 3rd, 7th, 28th and 56th days to examine the effect of changing bacteria dose on the strength and strength development of the hardened mortar samples. Compressive strength tests were carried out according to ASTM C109 standard, using 50 mm × 50 mm × 50 mm cube samples. 50 mm × 50 mm × 50 mm mortar samples were kept at room temperature in airtight sealed plastic bags for 24 hours in order to prevent the evaporation of the water contained. Then, the samples taken out of the molds were named and placed in airtight plastic locked bags and kept until the test day. On the test day, samples were taken out of locked bags and tested with a loading speed of 2 kN / s using a compressive strength test equipment (UTC-5700, Turkey) with a loading capacity of 300 kN. The tests were carried out on days 3, 7, 28 and 56. The strength values obtained in the bio-mortar samples were compared with the reference and control samples. At least three samples were used for each test, and mean values and standard deviations are presented.

3.2.4. Preparation of Bioconcrete Mixtures

In addition to the mortar samples, considering the recommendations made in the development report, bio-concrete samples containing two different doses of bacteria were prepared to examine their mechanical properties. Based on ACI 211 principles for one cubic meter of concrete mix, the designed concrete content is 966 kg / m^3 coarse aggregate, 870 kg / m^3 fine aggregate, 394 kg / m^3 CEM I 42.5R cement and 236.4 kg / m^3 tap water. in the form. The water / cement ratio was determined as 0.6, taking into account the amount of water added in terms of workability while preparing the reference mixture. In the mixture, aggregates with dmax = 16 were used as coarse aggregates, and aggregates varying in size between 4 mm and 12 mm were used as fine aggregates. It is aimed to obtain C25 / 30 properties in the reference mixture. The granulometry curve for the maximum aggregate size is given in Figure 3.3.

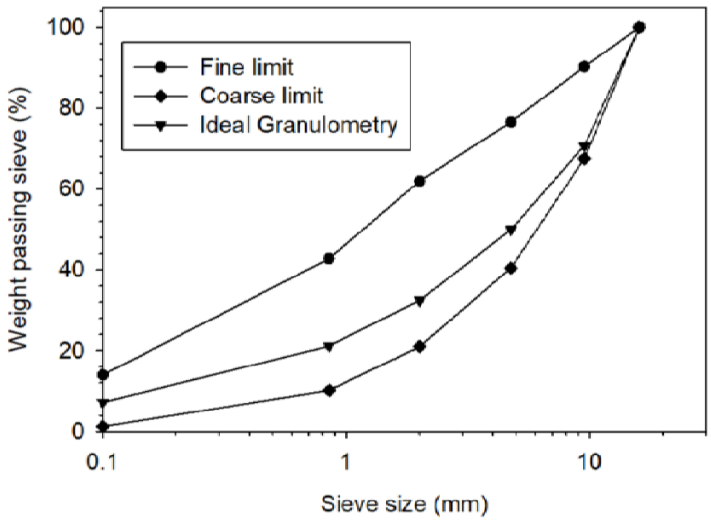

Figure 3. 3. Fine and coarse aggregate granulometry compared to the ideal granulometry curve.

The concrete mixture is prepared to be poured into 100 mm × 100 mm × 100 mm molds that are suitable in TS-EN-12390-1. 10 L of concrete was poured for each different mixture. The base concrete mixture was used as the reference sample and in addition to these, 5.00% calcium formate (19.70 kg / m^3) and 2.00% calcium nitrate (7.88 kg / m^3) were used in the control sample. In order to determine the upper and lower limit in bioconcrete mixtures, in addition to the control sample mixture, 0.25% bacteria (1.4 kg / m^3 biogranule) and 2.50% bacteria (14.1 kg / m^3 biogranule) are added by the weight of the cement and content of bioconcrete sample was prepared. Prepared concrete samples and their contents are given in Table 3.4. After the samples were poured into 100 mm × 100 mm × 100 mm molds, they were placed on the vibration table and placed at a frequency of 50 Hz (3000 rpm), then the molds were covered with a nylon for 24 hours at room temperature (Figure 3.4-a). After it was removed from the molds, it was taken into the water tank and cured until the test day (Figure 3.4-b).

In order to understand the workability of the samples in fresh concrete, the slump test was carried out in accordance with the EN 12350-2 standard. The workability of bioconcrete is compared with reference and control samples, taking into account the minimum 50 ± 25 mm and maximum 80 ± 25 mm sag values defined as the limit values for the reference sample in the EN 12350-2 standard. The compressive strengths of the hardened

bioconcrete samples at 28 and 56 days were compared to the reference and control concrete samples.

Table 3. 4. Tested bio concrete mixtures and their contents.

Concrete mixtures	Biogranule amount[1] (kg/m^3)	CF[2] (kg/m^3)	CN[2] (kg/m^3)	Coarse aggregate (kg/m^3)	Fine aggregate (kg/m^3)	CEM 42,5 R (kg/m^3)	Water (kg/m^3)
Reference	-	-	-	966.0	870.0	3940	236.4
Control	-	19.7	7.9	966.0	870.0	394.0	236.4
Bio-0.25%	1.4	19.7	7.9	966.0	870.0	394.0	236.4
Bio-2.50%	14.1	19.7	7.9	966.0	870.0	394.0	236.4

[1] Bacteria:Biogranule ratio 0.7 g/g.
[2] CF: Calcium formate. CN: Calcium nitrate.

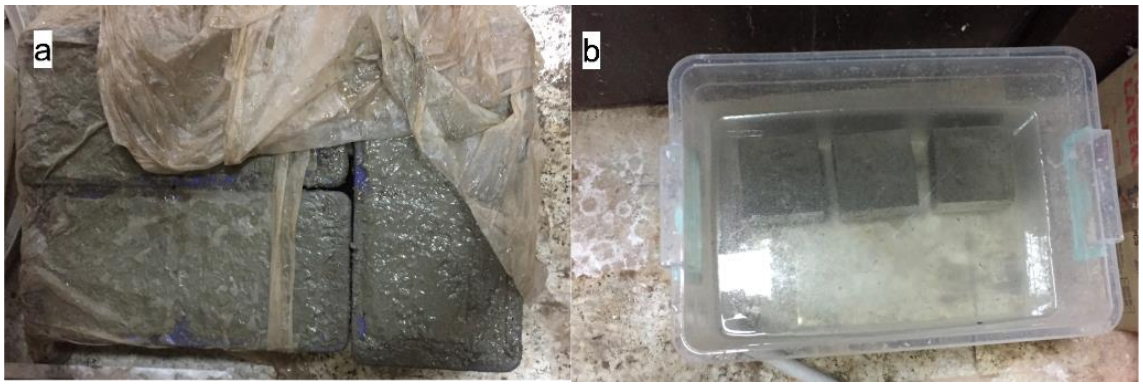

Figure 3. 4. Preparation of bio concrete mixtures in (a) 100 mm × 100 mm × 100 mm molds and (b) curing in the water tank until the test day.

3.2.5. Determination of Self-healing Capacity in Biomortars

The contents of the bio-mortar samples prepared for the self-healing capacity tests are given in Table 3.1. The mortar mixes prepared in accordance with the EN 196-1 standard were poured into 30 mm × 30 mm × 340 mm steel molds, with a ~ 500 mm long and 6 mm diameter ribbed steel reinforcement in the middle.

Molded mortar samples were separated from their molds after being kept in airtight sealed bags for 24 hours at room temperature (Figure 3.5-b). The steel reinforced mortar samples separated from the molds were named and placed in airtight plastic locked bags to prevent the evaporation of the water contained and kept at room temperature for 28 days (Figure 3.5-c). In this process, it was confirmed that curing was performed without evaporation

by monitoring whether there was evaporation with water in a container placed in sealed bags.

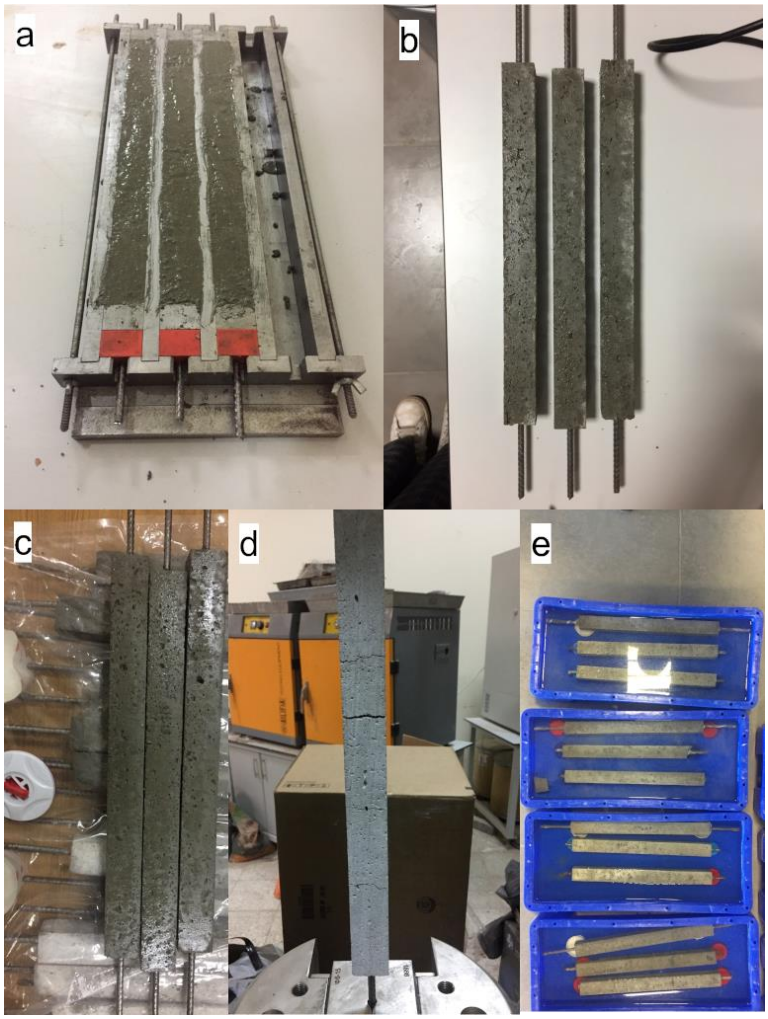

Figure 3. 5. Steel molds used in microbial self-healing bio-mortar tests (a) while preparing mortar samples; (b) samples of reinforced mortar removed from molds; (c) samples of reinforced bio-mortars stored in airtight sealed bags; (d) forming cracks in samples of reinforced bio-mortars; (e) curing of cracked samples of bio-mortars in water.

In order to perform self-healing tests, mortar samples were subjected to uniaxial tensile test at a speed of 0.01 mm/s on day 28, and many cracks of different sizes were obtained on the sample (Figure 3.5-d). Uniaxial drawing was performed by placing 80 mm ribbed steels outside on both sides of the mortar sample between the jaws of the pulling equipment (Figure 3.5-d). The cracks were viewed and analyzed under the microscope, and the crack widths at t0 were determined for each individual sample. The cracked samples were then cured in individual containers, completely immersed in tap water for

four weeks (Figure 3.5-e). The samples were taken out of tap water every week and left to dry for a while, and then the crack healing percentage was calculated using the formula given in Equation 5 by taking the images of the cracks via macroscope (Leica Z16 APO). At least 60 points were measured for each crack width and the healing performances of these 60 points were analyzed and average values and standard deviations were presented.

$$\text{Crack healing \%} = [1- (w_t / w_{initial})] \times 100 \quad (5)$$

w_t = crack width measured at any time t (days)
$w_{initial}$ = crack width before any improvement, at time t_0

In order to compare autogenous and microbial healing, the crack healing performances of the reference (sample containing only basic mortar components) and control (sample containing nutrients in addition to the basic mortar components) were examined in the same way with the biogranulated samples.

Samples with a crack healing efficiency of 90% and above within the scope of this completed thesis are defined as self-healing samples. Apart from that, in self-healing samples, the widest crack gap, which heals 90% and above and at the same time, in cracks narrower than itself, has an acceptable high percentage of closure, was determined as the self-healing capacity of the sample. The lowest bacterial dose as the minimum amount of bacteria required for biomortar has been determined among the tested bio-mortar samples which has a higher capacity than the autogenous healing capacity of reference and control samples in the same time, or repairs the specified autogenous-healing crack width in a shorter time compared to the reference and control samples, a cement composite that repairs itself by microbial means.

3.2.6. Statistical Analysis

At least three samples were used in all tests. The means and standard deviation values of the obtained results are presented. Results obtained with biobased samples were compared with reference sample and control sample using one-way ANOVA analysis and Holm-Sidak method, one-way ANOVA analysis among themselves and Fisher's LSD 5%.

4. RESULTS AND DISCUSSIONS

4.1. Biogranulation Process and Properties of Produced Biogranules

In the first stage of the thesis, biogranules containing active compact denitrification core were produced and quality tests of the produced granules were made. In the quality test, the dry size distribution of the granules was examined, the activity of the denitrifying center was measured, the water absorption amounts were tested and the tests for the compositions of the biogranules were carried out. In addition, the granules produced were viewed under microscope and scanning electron microscope, and the bacterial and mineral distribution in the granules was followed as a quality control parameter by element mapping. The variation of the parameters followed during the reactor operation is given in Figure 4.1.

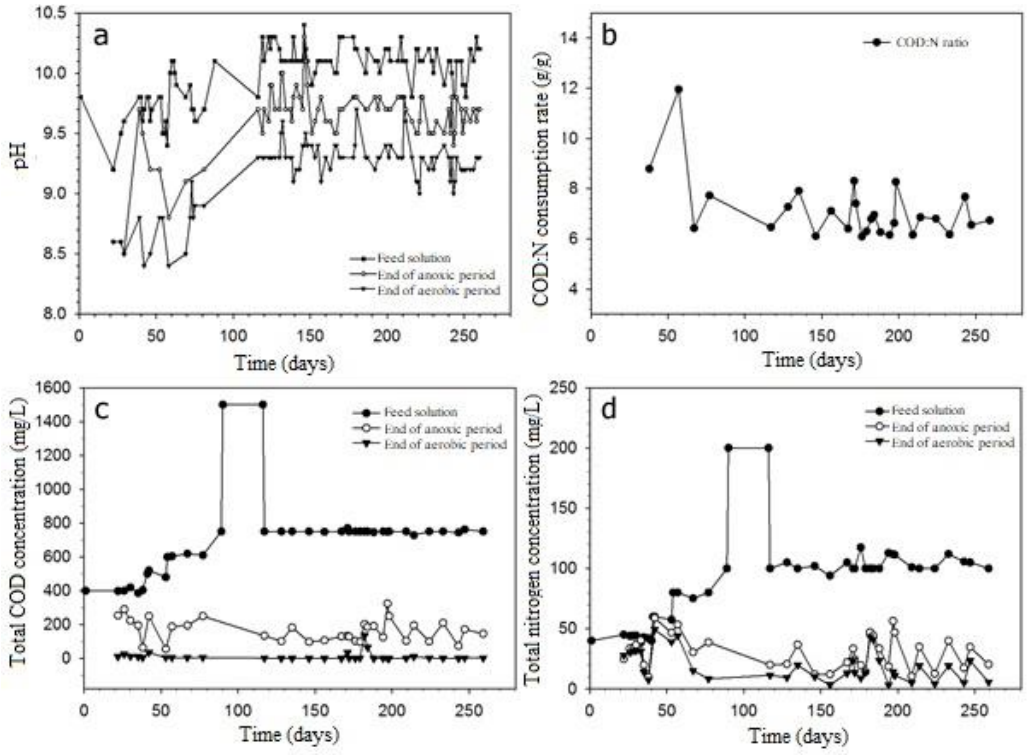

Figure 4. 1. Parameters monitored at different times in the feed solution and the reactor (a) pH; (b) the amount of COD removed per N; (c) change of COD; (d) change of total nitrogen.

Pasteurization of vaccine sludge taken from Kayseri Sugar Factory's advanced biological wastewater treatment plant ensured the elimination of microorganisms that could not

form spore from the initial microbial community. Therefore, the produced biogranules have no pathogenic feature. Since the pasteurization process reduces the amount of bacteria in the graft sludge, a lower concentration of nutrients was given in the first days of operation in order for the system to get used to the new operating conditions and for the growth of resistant microorganisms remaining in the sludge. The amount of food was increased with the increase in the amount of bacteria in the reactor. Initially, the nutrient solution was fed with a COD:TN ratio of 10.0, this ratio was gradually reduced to 7.5 during granulation and the granulation rate of bacteria reached equilibrium (Figure 4.1). The pH of the nutrient solution was kept around 10 while the biogranulation reactor was operated in equilibrium.

4.1.1. Biogranulation Process

The granulation process of the bacteria was continuously followed by 30 minutes and 5 minutes of sludge volume indices (SVI). The fact that the SVI is below 50 ml / g is the most important indicator of a granular system [109]. In addition, the SVI_{30} / SVI_5 ratio gives approximately the granule percentage of the sludge in the reactor[97] In the light of this information, it is seen that the amount of granules in the reactor started to dominate from the 69th day (Figure 4.2-b). With the increase in the dominance of the granules, a stable situation was obtained in nitrogen and carbon consumption. Only after the days when the granules were collected from the reactor, increases were observed in the amount of nitrogen in the effluent and the amount of carbon in the anoxic period effluent (Figure 4.1-c,d, Figure 4.2-a). This situation was caused by the deterioration of the nutrient/microorganism ratio in the reactor, and it returned to its normal trend with the proliferation of granules (Figure 4.1-c,d, Figure 4.2-a). The decrease in the amount of bacteria in the reactor below 3000 mg/L adversely affects the stability of the biogranule reactor in terms of granulation and microbial activity [97]. For this reason, while collecting the granules produced in the biogranule reactor, care was taken not to decrease the volatile suspended solids (VSS) value below 3000 mg/L. (Figure 4.2-a). The days when the produced granules were collected in a way that would have a minimum impact on the reactor performance are indicated in Figure 9a, and total suspended solids (TSS) and volatile suspended solids (VSS) changes within the reactor during the production period are also presented. In addition to the SVI_{30} and SVI_5 measurements, wet size distribution analysis was performed on a 750 mL sample taken from the reactor on the

130th day of operation, while the reactor was operating in equilibrium. In the literature, sizes up to 200 μm are classified as flock, and pellets larger than 200 μm are classified as granules[110] According to the results of the dimensional analysis, 89.5% of the reactor content consists of biogranules (Figure 4.2-c). The SVI_{30}/SVI_5 ratio, which corresponds to the same day, was measured as 85% (Figure 4.2-b). The similarity of the results of both analyzes once again demonstrated the usability of the SVI_{30}/SVI_5 ratio for monitoring the biogranule production reactor.

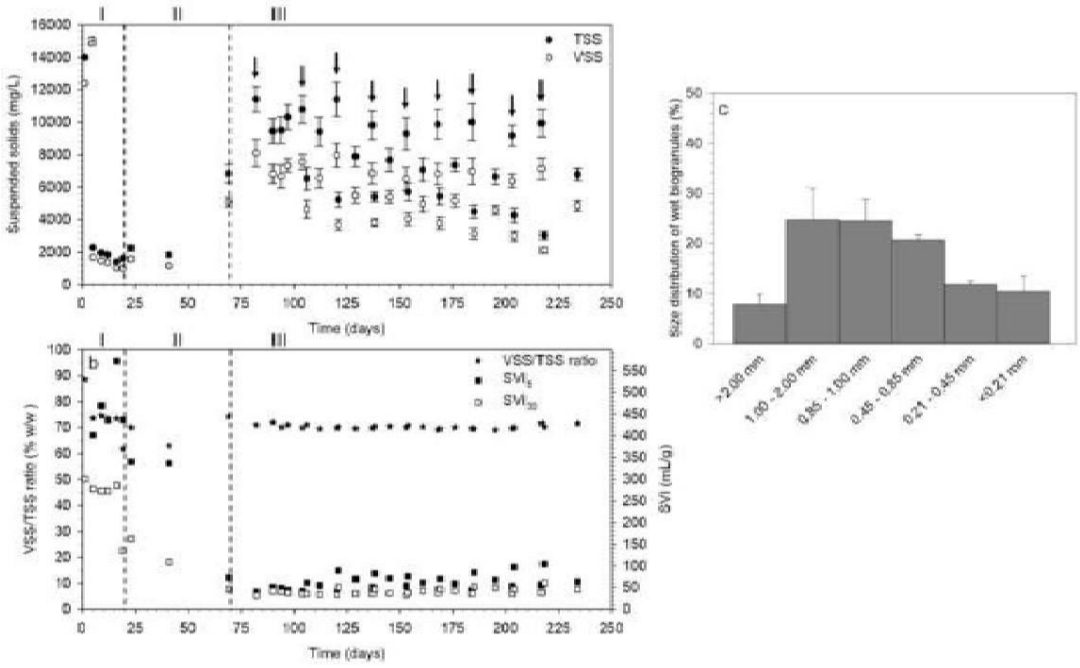

Figure 4. 2. Granulation parameters monitored during the acclimation (I), granulation (II) and production of biogranules (III) process. (a) suspended solid content (n=3); (b) SVI and bacteria/biogranule ratio; (c) average biogranule size distribution in reactor (n=5).

4.1.2. Activity and Quality Control of Biogranules Produced

During the quality control of the produced biogranules, the dry size distribution of the biogranules taken from the reactor was examined, the activity of the denitrified core was measured, the water absorption amount was determined and analyzes were made to determine the chemical composition of the layers in the biogranules.

Carbon oxidation through nitrate reduction is a process that takes place under anoxic conditions in the absence of dissolved oxygen. As can be seen in Figure 4.1-d, after the

completion of granulation in the bioreactor, a decrease in the amount of total nitrogen was observed in the aerobic period. Since nitrate is the only source of nitrogen fed to the reactor, the total nitrogen removed during the aerobic period also means nitrate reduction under aerobic conditions. Since the dissolved oxygen in the reactor cannot reach the center of the biogranules during the aerobic period, an anoxic zone is formed in the center of the biogranules and denitrification bacteria perform the carbon oxidation by using the nitrate reduction metabolic pathway [66]. Therefore, the total nitrogen removal in the aerobic period observed in Figure 4.1-d is also the most basic indicator that the biogranules have a compact denitrifying center. In addition to these observations, the denitrification activity of the granules during one cycle was measured by kinetic analysis, in which one cycle of the reactor was followed with 15-minute samplings. As seen in Figure 4.3-b, the NO_3^- and $HCOO^-$ ions (in COD mg/L) fed to the reactor were rapidly consumed during the anoxic period. In addition, NO_3^- reduction and a decrease in the total nitrogen content were observed in the first 45 minutes of the aerobic period, where the dissolved oxygen level ranged from 1.0 mg/L to 5.4 mg/L (Figure 4.3-b,d). This shows that there is an anoxic environment in the center of the granules and that the biogranules can oxidize the carbon source ($HCOO^-$) using NO_3^- even in the presence of oxygen in the environment.

Three collections of granules were made from the reactor. After each granule collection, the granules were dried at 60 C for 48 hours, and then the dry size distribution was determined by sieve analysis. Figure 4.4 shows the dry size distribution of the granules obtained from the reactor. Among the obtained dry granules, those smaller than 0.45 mm and larger than 2.00 mm were added back to the granule reactor, and dry granules between 0.45 and 2.00 mm were separated for use in the tests of the mortar samples. The amount of granules re-added to the reactor corresponds to approximately 34% of the total amount of granules produced (Figure 4.4). In the first stage, the biggest part, approximately 30%, of the granules produced during the operation were biogranules between 1.00 and 2.00 mm (Figure 4.4). This ratio corresponds to approximately half of the granule amount (0.45-2.00 mm) that passed the size tests to be used in bio-mortar tests.

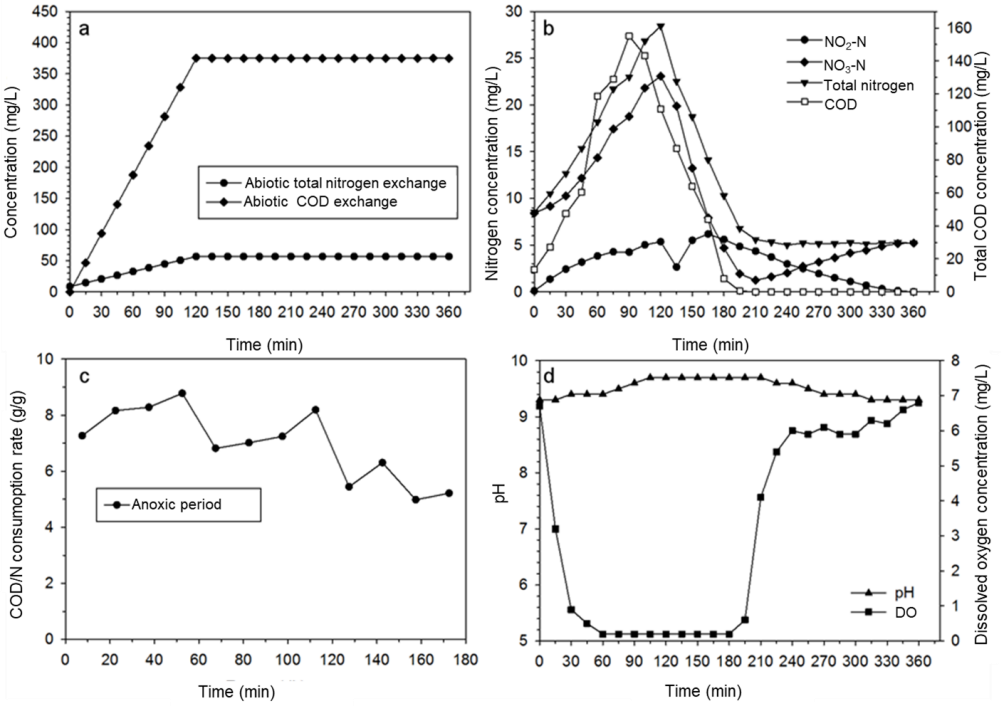

Figure 4. 3. Kinetic examination of the new cycle following a healthy cycle under stable conditions with 15-minute samples (a) change of COD and TN in the reactor under abiotic conditions; (b) COD and TN exchange within the biogranule reactor; (c) the amount of COD consumed per weight N in the biogranule reactor during the anoxic period; (d) pH and DO change in the biogranule reactor. Abiotic change has been achieved by theoretical approach.

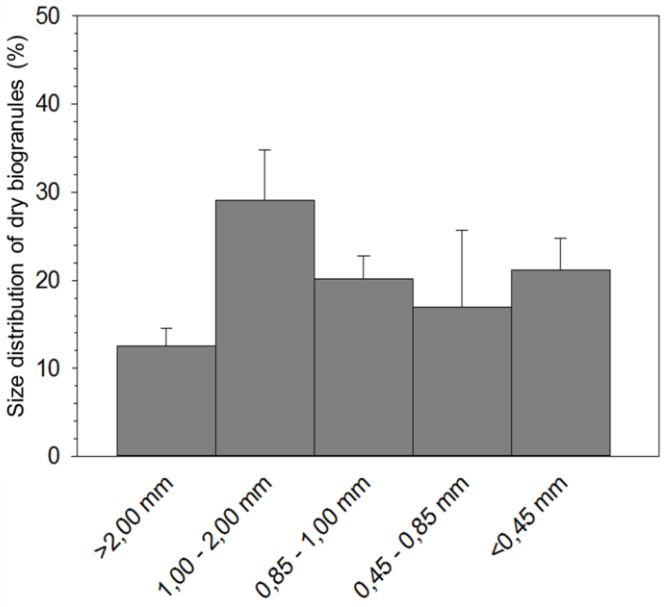

Figure 4. 4. Size distribution of biogranules obtained from the biogranule reactor after drying. The mean of the sums at different times is given, with error bars showing the standard deviation (n=10).

1 hour and 24 hour water absorption amounts of biogranules produced and separated for use in experiments by sieve analysis with a size between 0.45 and 2.00 mm were tested. Accordingly, it was determined that biogranules absorb 16 ± 8% of their weight at the end of 1 hour and 20 ± 8% of their weight at the end of 24 hours (Figure 4.5). According to the results, it can be said that the amount of water absorbed by biogranules in 1 hour does not show a statistically significant difference from the amount of water they absorb in 24 hours, and the biogranules take the water they will take from the environment within 1 hour from the moment they come into contact with water.

According to the ASTM C191-04 standard, it is known that the set start of the mortar samples should be 60 ± 12 minutes at the earliest. In this case, in this thesis, it can be said that a quantity between 0.06 g and 3.80 g of water used in the mixture can be absorbed by the biogranules, and this amount does not significantly change the water: cement ratio.

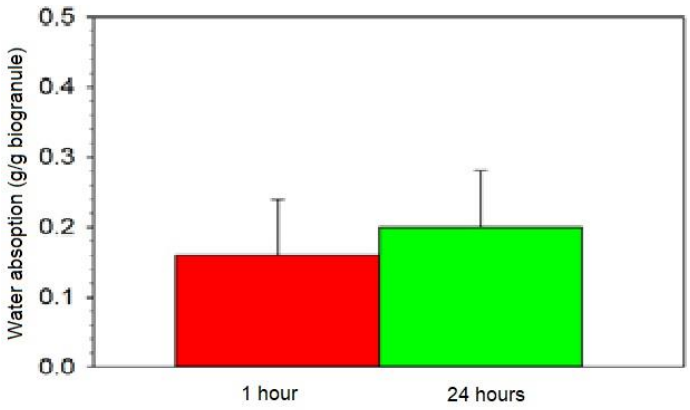

Figure 4. 5. 1 hour and 24 hour water absorption amounts of biogranules with a size between 0.45 - 2.00 mm used in bio-mortars. Error bars indicate standard deviation (n = 3).

During the production of biogranules, a nutrient solution and reactor operation was applied to form $CaCO_3$ and $Ca_3(PO_4)_2$ precipitates on the outer part of the granules. It has been reported in our previous studies that these minerals protect the biogranules against the pressure created by the narrowing pore widths with the hydration of the mortar and increase the survival rate of spores [66] The VSS/TSS ratio of the granules obtained in this thesis was measured as 70±1% (Figure 4.2-b). Therefore, 30% of the granules consist of inorganic substances. The chemical composition of the obtained biogranules was

determined by Fourier transform infrared (FTIR) analysis. The FTIR spectra of the biogranules obtained at different collection times are given in Figures 4.5-4.7. Accordingly, the presence of water and amine groups (3271 – 3600 cm^{-1} OH ve NH peaks), proteins providing rigidity (1646 cm^{-1} amide peak found in secondary proteins) and polysaccharides (2000-2300 cm^{-1} carbonyl peaks, 1033 cm^{-1} carbohydrate peak), calcite from inorganic minerals (1408 cm^{-1}, 871 cm^{-1}, 713 cm^{-1} carbonate peaks), aragonite (699 cm^{-1} carbonate peaks) and calcium phosphate (586 cm^{-1} phosphate peak) were determined in the granule. The absence of a significant difference in the peaks obtained in FTIR analyzes with biogranules collected at different times indicates that the chemical composition of the biogranules that are produced continuously and collected at different times are similar and the continuity of the production reactor is ensured. Biogranules obtained as a result of FTIR analyzes performed at different times were confirmed to contain $CaCO_3$ and $Ca_3(PO_4)_2$ as planned (Figure 4.6-4.8).

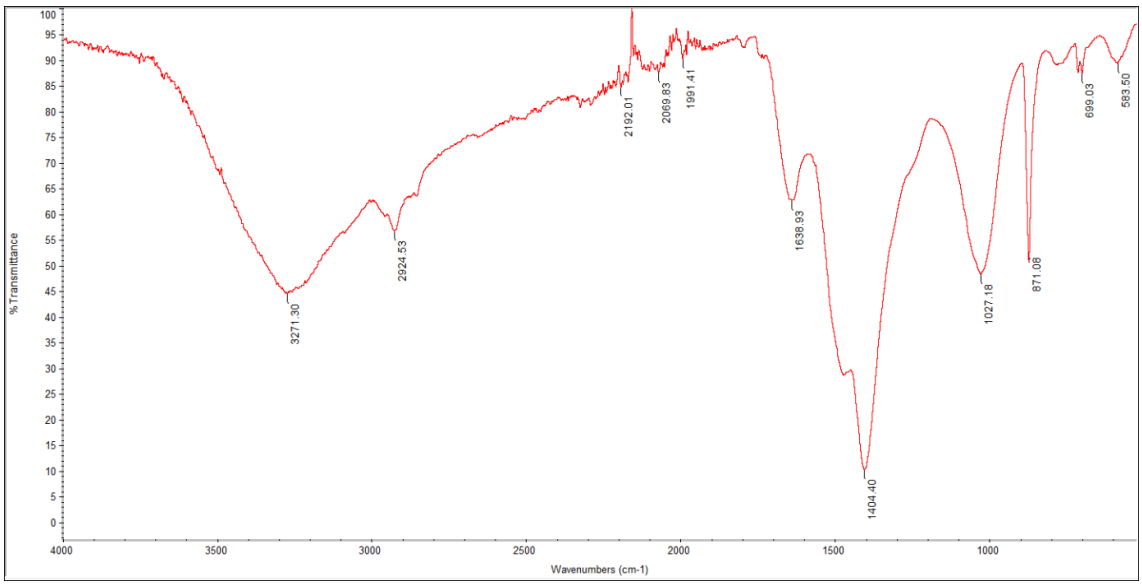

Figure 4. 6. FTIR spectrum of granules from the first harvest.

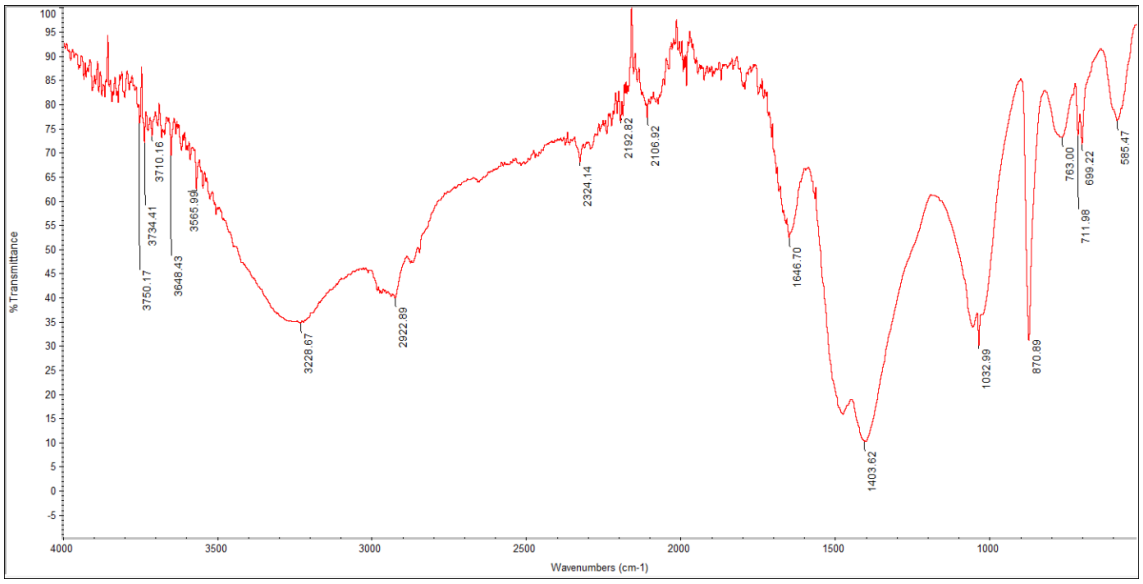

Figure 4. 7. FTIR spectrum of granules from the second harvest.

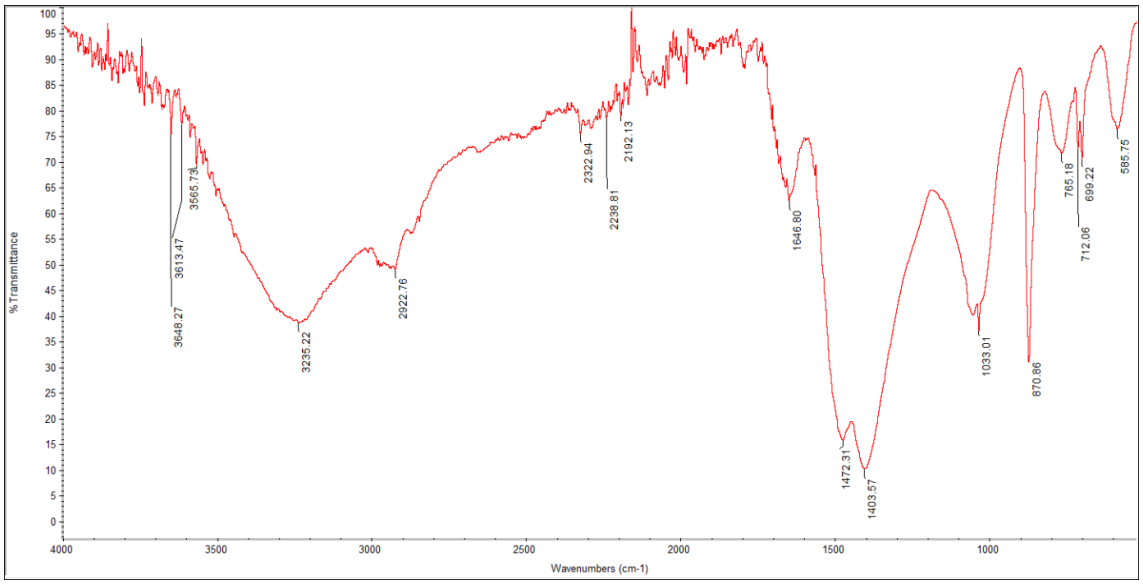

Figure 4. 8. FTIR spectrum of granules from the third harvest.

It is important that biogranules contain $CaCO_3$ and $Ca_3(PO_4)_2$, as well as in which layer of biogranules these minerals are formed in terms of biogranule quality. Biogranules were first visualized under a light microscope in order to understand where the deposits were formed. In addition, random samples taken from the produced biogranules were imaged under the scanning electron microscope (SEM) combined with energy dispersive X-ray spectroscopy (EDS) and the distribution of the minerals in the biogranules in the granules was determined by element mapping.

In Figure 4.9, the images of the wet and dry states of the biogranules obtained under the light microscope are presented. As seen in Figure 4.8-a,c, the middle part of the granules has a structure that is more compact and less permeable to light, while the outer part is less sparse and consists of a structure in which precipitate crystals can be selected. It can be said that the biogranules obtained mostly have an oval shape in both wet and dry form (Figure 4.9-c, d). It is seen that the outer part of the biogranules obtained is whiter and the inner part is darker (Figure 4.9-e). When the view of a dry granule divided into two is examined under the microscope, it is seen that its center is darker and its outermost layer is whitish (Figure 4.9-f). In the biogranules and granular sludge literature, it is stated that biogranules produced under oxygen-free conditions are black [112], while biogranules produced in an oxygenated environment are cream colour [97]. Therefore, the biogranules obtained are mostly dark in colour and the center is black, indicating that the granules consist of bacteria that grow under both aerobic and anaerobic conditions rather than aerobic bacteria, as expected from the reactor operating conditions in the thesis, and that the compact denitrification center is obtained in the center, which is active under anoxic conditions. It can be said that the outermost white layer consists of $CaCO_3$ and $Ca_3(PO_4)_2$ minerals.

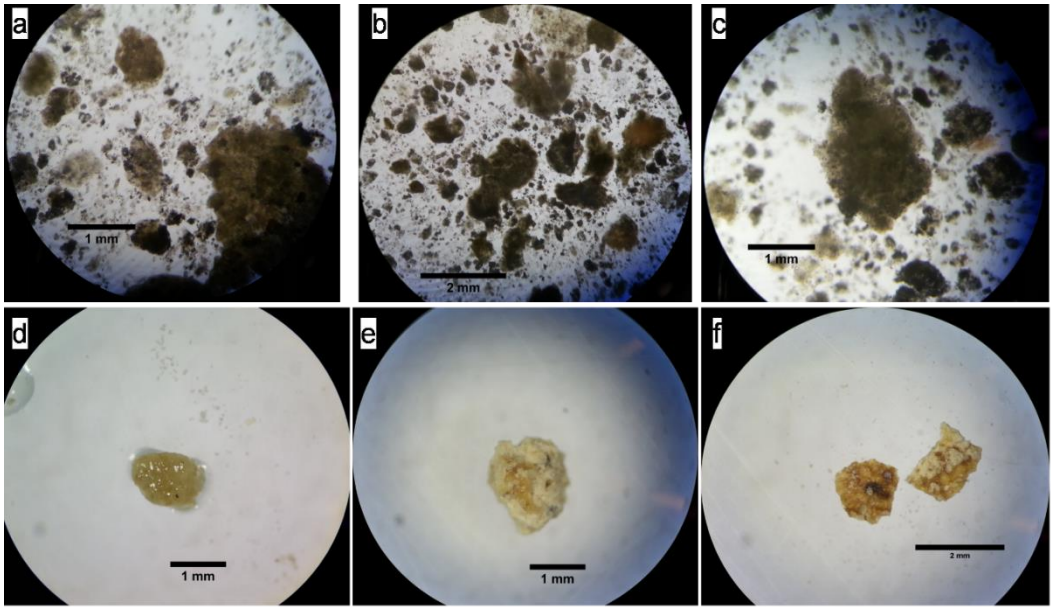

Figure 4. 9. Images of the wet state (a-d) of the biogranules produced in the biogranule reactor under the light microscope; e) the image of a dried one; f) the image of one divided in two.

Images and element maps obtained under the scanning electron microscope (SEM) combined with energy dispersive X-ray spectroscopy (EDS) verify the information obtained under the light microscope. Figure 4.10 shows the weight of P element in the inner layers of the granules divided into two, and the dominance of Ca and O elements with P in places in the outer layer. P is an element abundantly found in the structure of bacterial cells and in the protein layer of spores. There are also abundant in denitrification bacteria that store polyphosphate. On the other hand, Ca and O indicate $CaCO_3$ minerals that are densely found in the outer layer.

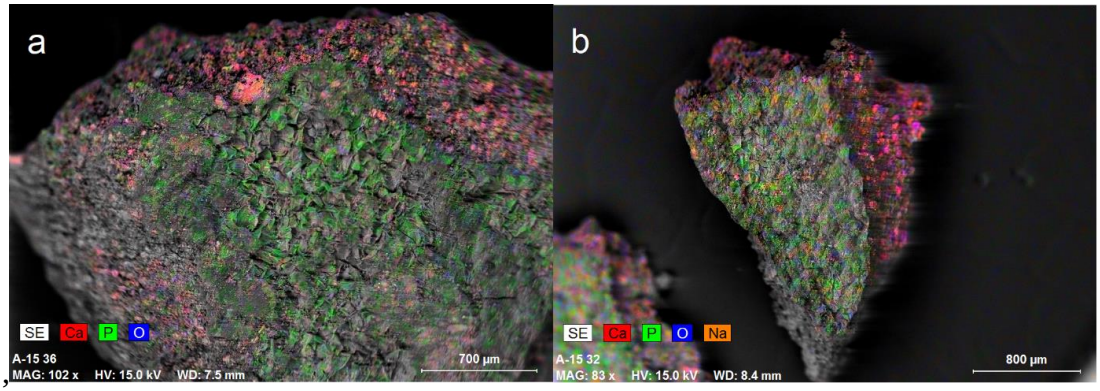

Figure 4. 10. The distribution of Ca, P, O elements in the produced biogranules as an indicator that the minerals are in the outer layer and the bacteria in the inner layer, (a) sample number one (b) sample number two.

In addition, bacterial spores in the contents of the dried and divided biogranules were also visualized under a scanning microscope (Figure 4.11-a, b). The dominance of P and O elements stands out in the section where the spores are located (Figure 4.11-c, d). This indicates that the bacteria in the granules are likely to be denitrifying bacteria that accumulate polyphosphate. The fact that no vegetative bacteria were found in the micrographs obtained indicates that the produced biogranules consist of spore-forming bacteria that can survive for a long time in the concrete and do not contain vegetative pathogenic bacteria.

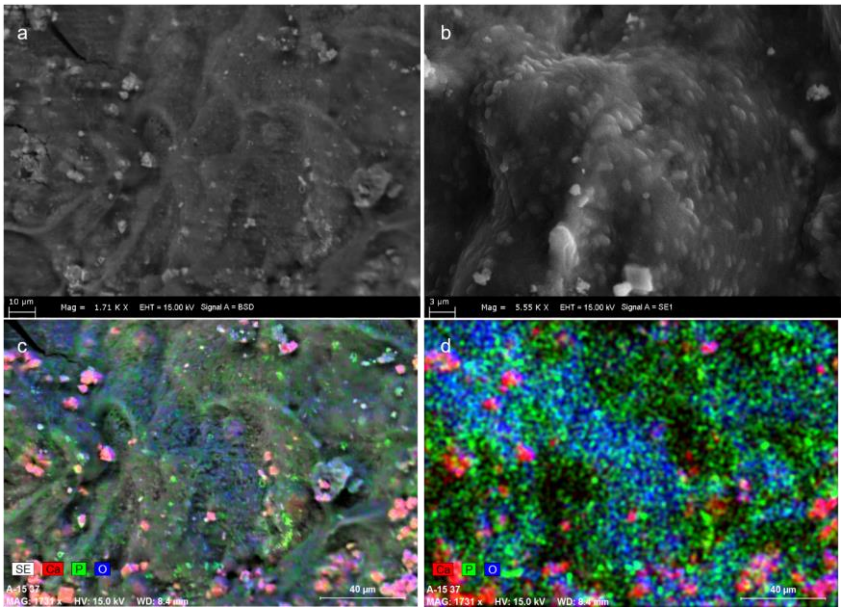

Figure 4. 11.From the inner layer of biogranules; (a) image of part of the section containing spores; (b) the view of the spores; (c) the section where the spores are located and the distribution of Ca, P and O elements in that section; (d) only Ca, P and O map of the section where the spores are located.

When the results are evaluated in detail, it can be said that the biogranules produced within the scope of the thesis are biogranules that trigger $CaCO_3$ precipitation, oxidize the carbon source through nitrate reduction in both oxygen and non-oxygen environments, and contain calcium salts in the outer layer that can protect the biogranules against the harsh environmental conditions formed in cement composites. In previous studies, it has been shown that the produced biogranules do not show activity in environments such as fresh mortar mixture where pH values reach 13-14, the spores do not revive during the mortar mixture, and the bacteria added into the mortar in the form of biogranules can even heal the cracks formed after 6 months [66], [68]. Although 6 months is a short period for cement composites, considering that the life cycle of a bacterium is 7 days on average, the stability of spores in a nutrient-free, waterless and alkaline environment over a period of 6 months indicates that it can remain stable in cement composites for longer periods until cracks form. Thus, it has been reported in the literature that spores survive up to 200 years under very extreme conditions [113]. Therefore, it was decided that these biogranules can be used as microbial additives in the experiments to be carried out with bio-mortar samples within the scope of the thesis, considering the activity and quality tests of the biogranules produced within the scope of

the thesis, without the need for a study about how long the produced biogranules can survive in cement composites. The produced biogranules were stored in plastic storage containers at room temperature to be used in mortar samples (Figure 4.12).

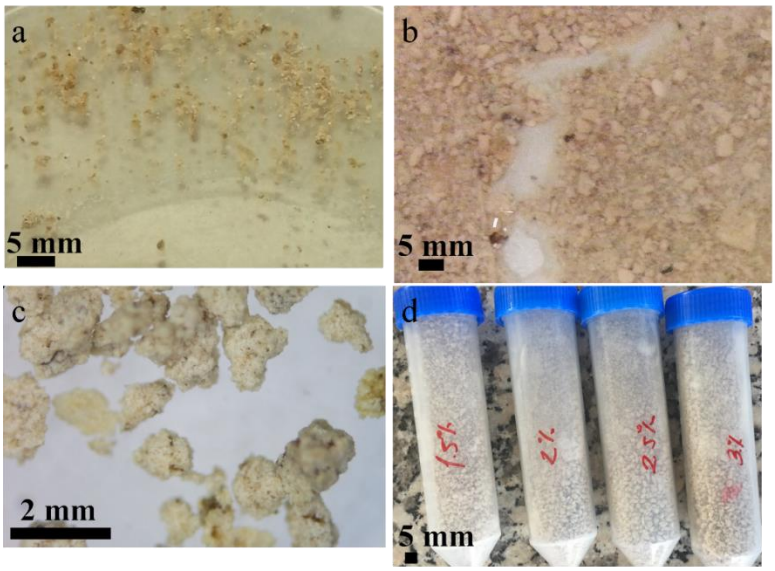

Figure 4. 12. The views of the granules used in the mortar sample (a) dried biogranules, (b) biogranules collected from the reactor and placed on oily paper to be dried, (c) non-dried mineral coated biogranules in the range of 1.00 - 2.00 mm, (d) size distribution biogranules ready to be added to the mortar mix, adjusted and placed in plastic tubes.

The use of minimum nutrient medium lacking in micronutrients, trace elements and vitamins successfully resulted in biogranules in approximately two months. The reactor size distribution indicated that 90% of the microbial culture consisted of biogranules which is parallel with Beun' study [110] (Figure 4.2-c). A second indicator of granular biomass abundance was described as the SVI_{30}/SVI_5 ratio [108]. When these two values for the same day in this study, Day 104, were compared, it was discovered that both the particle size distribution test (89%) and the SVI_{30}/SVI_5 ratio (85%) can be used interchangeably to evaluate the granular biomass inside the biogranule reactor. One of the most significant advantages of biogranules is their ease of separation from the liquor due to their superior settling properties. The SVI_{30} value has been reported to be an important indicator of granular sludge reactors, and the bacterial culture should be as compact as 50 ml/g [108]. During the entire granulating process and the granule production, as SVI_{30} was continuously monitored in this study, the settling properties

were significantly altered immediately following each harvesting of granules. The reduction was primarily attributed to the collection of larger granules. Nevertheless, a relatively short 2-week healing may be achieved, indicating that abundant biogranules that reduce nitrate in granular biomass can be harvested every 2 weeks. Dry biogranules smaller than 0.45 mm, corresponding to 34% of harvested biogranules, have been returned to the bioreactor after harvest. As a result, the bioreactor's net useful biogranule production yield was calculated to be 0.03 g biogranule.g^{-1} $HCOO^-$ and 0.70 g biogranule.g^{-1} NO_x-N. The bioreactor's gross biomass yields were 0.05 g biogranule.g^{-1} $HCOO^-$ and 1.07 g biogranule.g^{-1} NOx-N. Inside the reactor, the average granule size was 0.950±20 mm (Figure 4.2-c). The yields and abundant size of granules within the reactor were comparable with those reported earlier for the 4 hours cycle time-8 hydraulic retention granular sludge reactor [114], [115]. It is promising to further examine the cost-effective production of healing agents in higher proportions if such yields are achieved under minimal nutrients conditions. A paper assessed that the high intensity aerobic granular sludge reactor has a 5-fold greater yield of biomass, but that the abundance of granular size varies from 0.12 mm to 0.35 mm [116]. It is known that the compactness of biogranules is important for stability and easy separation between liquid media and that granules are primarily associated with the biomass growth rate, which leads to either abundant flocculant granular sludge or large fluffy granules in hight of biomass [114], [117]–[119]. As a result, if someone takes into consideration obtaining smaller size biogranules, the rate of biogranule production can be increased in accordance with sectoral demand. Achieving big biogranules (> 2 mm) comparable to those observed in several other granulation studies is not suggested for concrete application since they are fluffy, weaker against shear stress, and easily disintegrate due to nutrient limitation at the core [119]–[123].

In this thesis, kinetic analysis shows that the sizes of the biogranule are sufficiently small to allow nutrient access to the core, but large enough to avoid the diffusion of oxygen in the core. Oxygen diffusion was described as 0.2 mm to 0.4 mm in the microbial flocs [124]. The distribution of the particle size demonstrated that 68% of the biogranules were greater than 0.45 mm and that the average granule sizes were 0.950±0.20 mm. Kinetic testing further confirmed that the core of biogranules is reduced by compact nitrate as NO_x-N, while DO levels ranged from 1 mg/L to 6 mg/L. Even for the aerobic granular consortium, similar findings were reported earlier and the process was called

simultaneous nitrification and denitrification [118], [125]. The visual observations were used as another predictor for a denitrifying core. It is recognized that biogranules grown under anaerobic conditions seem to be dark, even black [112], whereas biogranules grown under aerobic conditions are lighter in color [97]. The color pattern indicates an entirely anoxic zone at the core, that allowed for the advancement of a denitrifying core community. It is therefore reasonable to assume that the biogranules can be obtained with a compact denitrifying core.

The kinetic analyses revealed that NO_x-N reduction occurred even in the absence of $HCOO^-$, despite the fact that the average [COD]:[N] consumption ratio was found to be 5.3 during the anoxic period (Figure 4.3-c). The observed behavior has been related with the feast/famine operation of the biogranular production reactor, that obliged some nutrients to be used by micro-organisms ($HCOO^-$ in this case) as internal polymers (polyhydroxyalkanoates-PHA) for further usage in famine conditions, to be stored within a microbial cell during the feast [125]. In several research findings PHA [38,39] were found to be the carbonyl peaks observed in 1730-1740 cm^{-1} in FTIR analyzes, and similar peaks for the dry biogranules were also noted in this thesis [126], [127]. Either glycogen or polyphosphates that accumulate organisms may be preceded by this metabolism. While the microbial species within the biogranules produced were not further experimentally identified, it can said that there were significant amounts of P and O in the core biogranules, due to the elemental mapping of the cross section of a biogranule. From these data, we suspect the polyphosphate accumulated by nitrate-reducing bacteria could be a percentage of the microorganisms in the core. Furthermore, in our earlier findings, it has been revealed the possibility for dry biogranules to resuscitate, grow and decrease nitrate without external PO_4-P [68], that could also indicate that certain polyphosphates are present in the core of the produced biogranules most probably because of the presence of phosphate accumulating nitrate reduction. In earlier studies investigating aerobic denitrification, similar observations like reduced denitrification rate of bacteria and restricted denitrification in the aerobic period were also identified [118], [125].

4.2. Characteristics of Fresh Biomass Samples and Maximum Usable Bacterial Dosage

Setting and flow tests were applied on fresh mortar samples. Based on the limit values in ASTM C191-04 and NBN EN 1008 standards, acceptable minimum values for setting start and end are determined as 60 minutes and 90 minutes, respectively. In the ASTM C191-04a standard, the standard deviation values for the setting starts between 49 and 202 minutes and the setting end between 185 and 312 minutes in the setting tests of hydraulic cements performed in a single laboratory with a single operator are stated as 12 and 20 minutes, respectively. Since there is no deviation determined in the standards regarding the setting values of cement mortars under the same conditions, the statistical significance of the differences (p = 0.05) was interpreted by using the same deviation values within the scope of this thesis. Setting test results are given in Figure 4.13.

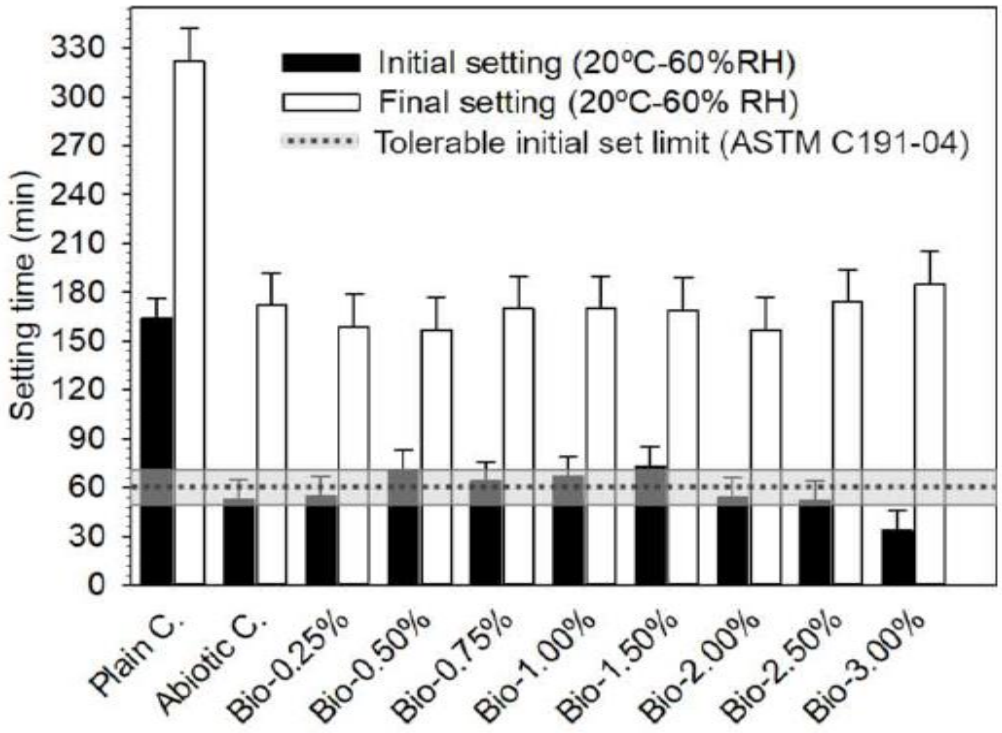

Figure 4. 13. Setting start and end setting times of mortar samples with different content tested. (-) minimum acceptable setting start-up time.

It has been found that the setting starts and set ending times of the control mixture and the bio-mortar mixture are completely different from the reference mixture. Setting start of the control mixture takes place within 55 ± 12 minutes, and ending of setting takes

place within 172 ± 20 minutes (Figure 4.13). Since the control mixture has only nutrient content and does not contain bacteria, this change in setting times is entirely due to 5.00% calcium formate and 2.00% calcium nitrate by weight of cement added to the mortar mixture. These results are consistent with the set starting (65 ± 12 minutes) and setting ending (146 ± 20 minutes) times observed by thesis co-advisor in previous study for the same mixture [67].

The main purpose of the fresh mortar tests conducted within the scope of this completed thesis is to determine the maximum amount of biogranules that can be used in the mortar content in order to obtain cementitious composites with microbial properties similar or better than the control sample, within the limits specified in ASTM C191-04 and NBN EN 1008 standards. Therefore, the fresh mortar properties of the samples containing bacteria in the form of biogranules in different doses were primarily compared with the control sample. In contradictory cases obtained in different fresh mortar experiments, comparisons with the reference sample were made and evaluations were made.

It was determined that bacteria in the form of biogranules added to the control mixture up to 2.50% by weight of cement did not cause a significant change in setting start and set ending times compared to the control sample (Figure 4.13). When this dose was increased to 3.00%, it was observed that there was a significant decrease in the starting time of setting. The setting start time of the Bio-3.00% mixture was measured as 34 ± 12 minutes, well below the acceptable 60 minutes limit (Figure 4.13). Regardless of the bacterial dosage, the expiration time of the bio-mortar mixes was similar to that of the control sample (Figure 4.13). In the previous studies of the thesis co-advisor, it has been shown that the bacteria in the form of biogranules added at a dose of 0.50% by weight of cement do not affect the setting time of the biomortars [88]. The findings obtained in this thesis show that it is possible to add 2.50% of bacteria by weight of the cement in the form of biogranules to the mortar without causing a significant change in the setting time of the mortar.

The set start and end set times obtained in the control sample are considerably lower than the cement composites used in the field. However, since these durations are within the acceptable range specified in ASTM C191-04 and NBN EN 1008 standards, no changes were required within the scope of the thesis. In previous studies, the amount of nutrients

required to be used in the content of self-healing concrete has been optimized based on the amount of nutrients that can be used by bacteria in case of cracks [67]. In the study, calcium formate and calcium nitrate content were tested in 12 different compositions ranging from 0.63% (CF) and 0.25% (CN) by weight of cement to 7.50% (CF) and 3.00% (CN). Although it is determined that it is disadvantageous compared to other mixtures in terms of setting start and end times, it has been determined that the most advantageous mixture in terms of the amount of nutrients that can be used by bacteria after cracking is the mixture containing 5.00% calcium formate and 2.00% calcium nitrate by weight of cement. For example, the setting start time of the mixture containing 2.50% calcium formate and 1.25% calcium nitrate by weight of cement was reported as 108 minutes and setting completion time as 173 minutes [67] However, at this dose, the amount of nutrients in the environment in the first 4 weeks after crack formation decreases by half compared to the optimized mixture [67] Therefore, it is possible to observe decreases in the speed of self-repair and self-healing capacity while extending the setting time by reducing the amount of nutrients in the control mortar mixture. Considering this situation, changing the amount of nutrients in the control sample was excluded from the scope of this thesis in order not to experience any nutritional problems in the experiments regarding the determination of the self-healing capacity. In the light of the results of the completed study, it seems possible to make modifications in the setting time with a nutrient amount optimization by keeping the bacterial dose constant.

Additionally, microbial self-healing concretes definitely need water to repair themselves. Therefore, it is planned to be used mostly in structures such as bridges, underwater structures and tunnels. It is thought that cement composites produced in this thesis, which have a short setting start time and which repair themselves with microbial means, will be used in the field, since these types of structures can be used, such as pre-fabricated concrete structural elements, where short setting times are not a problem.

Another option regarding setting times is the use of set retardants and super plasticizers. Since the effects of set retardant materials and plasticizer additives on biogranules and on the self-healing capacity of bio-mortar are not known, this option has been chosen only for use in conditions of poor setting and workability that would hinder the progress of the study. In the literature, when 0.50% by weight of cement was used in the form of bacteria in the form of biogranules, it was possible to obtain cement composite that repairs itself

by microbial means [66]. Within the scope of this thesis study, it was determined that bacteria up to 2.50% by weight of cement can be added to fresh mortar samples. On the other hand, set retarding materials and plasticizer additives pose a greater risk to the progress and success of the study, as they may have a negative impact on the activity of biogranules due to the aforementioned obscurity. In order not to take such a risk, this option has not been evaluated within the scope of this thesis.

With the earlier findings, a mortar mixture similar to Bio-0,50% was investigated, this thesis study also revealed reasonable results [88]. The research reveals no big difference in the setting time for the mortar mixture as a benefit of that culture as compared to axenic cultures, with the addition of 0.50% bacteria w/w cement together with 3.00% calcium nitrate (CN) and 2.00% calcium formate (CF). The total setting times documented in this research were however much shorter than the results obtained earlier. The larger nutrient content of the recently developed mix was ascribed to such a decline in setting times based on optimal nutrient content [67]. The initial (65±12 min) and final (146±20 min) setting times reported were around the same as those reported by that study which tested 5.00% CF and 2.00% CN for fresh mortar properties abiotically. Such resemblances revealed that nutrients, namely calcium and calcium formates, are the cause of the distinction between the plain mortars and all other test mortar mixtures. The nutrients used are commercial products concrete admixtures, which were used to increase the resistance to freezing-thawing and to speed the setting [128], [129]. Since a combination of the two set accelerators in this thesis has been used, a considerable reduction has become in the initial setting time. While decreasing the mix's CF and CN contents can increase the initial and final setting times to 110 min and 170 min, it is necessary to admit that such a nutrient content decline leads to a significant reduction in microbial crack healing nutrient availability [67].

Findings from fresh mortar properties helped to assess the ability of the biogranules to protect themselves and the interplay between the spores and the cement matrix. Earlier research examining the direct incorporation of bacteria/spore into mortar mixtures have shown that a set delay occurs because the bacteria simply have a contact with the organic matter and the cement matrix [88], [130]. When bacteria died in the concrete's highly alkaline environment, the organic content of bacteria was left into the fresh mortar mixture and causes set retardation. According to Jonkers et al. [131], spores can be

completely or partially lost during the mixing, hardening, and subsequent shrinkage of pores. The protein coating of the spores is unveiled upon loss, causing foaming and interfering with the rate of hydration process, significantly delaying the initial setting. Furthermore, in a research done by the thesis co-advisor, it was discovered that when an unprotected denitrifying microbial culture, *Diaphorobacter nitroreducens*, was introduced to mortar at a dose of 0.50% w/w cement, both initial and final setting times risen by about 40 minutes [88]. The outcome of the research, showed that integration of biogranules (with EPS and mineral layer) prevented a possible interaction between the cement matrix and the organic material, and hence no retardation on set was recorded, even at high dose bacteria (e.g. Bio-3.00%).

Flow table tests were carried out in accordance with the NBN EN 1015-3 standard. The maximum acceptable deviation between the tested samples and the reference sample was determined to be 10%. Workability and consistency evaluations were made according to the results obtained from the flow table test. Flow table results are presented in Table 4.1. First of all, the flow values of the different mixtures were compared with the flow value of the plain mortar mixture (reference). Accordingly, it was observed that even the flow of the Bio-3.00% mixture, which has the lowest flow, remained within the 10% deviation limit. Then, the flow values of the bio-mortar samples were compared with the flow values of the control mixture. According to this comparison, since the flow value of the Bio-3.00% mixture corresponds to 89% of the control flow, the Bio-2.50% mixture has been determined as an acceptable upper limit. However, problems were experienced during the settlement of both Bio-3.00% and Bio-2.50% mixtures (Figure 4.14). As a result, although the flow values of both mixtures were acceptable, it was concluded that the workability was low, and it was decided to evaluate the upper limit value by considering the compression strength results. From the comparisons made, it can be said that the comparison made with the flow of the control mixture gives more meaningful results regarding the effect of the bacterial dose. Because, in comparison with the control mixture, the effect of the added biogranules on the flow, workability and consistency is revealed directly. The flow results revealed that the Bio-2.50% mixture can be used as the upper limit, adding more biogranules causes changes in the mortar rheology, decreases the workability and thus negatively affects the settlement of the mortar.

Table 4. 1.Comparative flow values of mortar samples with different content tested.

Mortar mixture	Flow (mm)	Flow (Compare to R %)	Flow (Compare to C%)
R	118	100	98
C	121	103	100
Bio-0.25%	116	98	96
Bio-0.50%	124	105	103
Bio-0.75%	125	106	103
Bio-1.00%	123	104	102
Bio-1.50%	123	104	102
Bio-2.00%	121	103	100
Bio-2.50%	113	96	93
Bio-3.00%	108	91	89

[1]R: Plain mortar used as a reference, C: Control mixture containing 5.00% calcium formate and 2.00% calcium nitrate by weight of cement.

The percentages given in biomixtures are the amount of bacteria by weight of cement.

Figure 4. 14. Extremely porous outer surface thought to be related to the settling problem when Bio-2.50% and Bio-3.00% mixtures are removed from the molds.

It is known that there is a delay in setting times when organic substances come into contact with the fresh mortar matrix. Bacteria are also organic substances and when bacterial cells, especially protein-rich spores, undergo lysis, the organic substances in the cell structure are released and come into contact with the mortar matrix and cause delays in setting time [88], [130]. For example, Diaphorobacter nitroreducens cells added to the mortar mixture in vegetative form at a rate of 0.50% by weight of cement underwent lysis,

causing a 40-minute increase in both the set starting and setting ending times [88]. However, in the study conducted within the scope of this thesis study, it has been observed that high doses of biogranules can be added to the mortar mixture and this does not delay the setting time of the mortars.

Bacteria added to the content of bioconcrete generally undergo lysis due to the forces that occur during the mortar mixture, the high temperatures during setting and the basic environment, and the narrowing of the pore widths during advanced hydration [88], [131], [132]. In this thesis, the findings obtained so far show that when bacteria are added in the form of biogranules, it does not cause a delay in setting time up to 3.00% by weight of cement. Since there is no delay in setting, it can be said that bacteria do not come into contact with the mortar matrix during the setting period. In this case, it would not be wrong to say that the protective layer around the biogranules produced within the scope of the thesis, which consists of low-solubility calcium salts, protects bacteria and prevents lysis both during the mortar mixture and during the setting period. Since the spores in the produced biogranules cannot show any vitality and activity even if they are in contact with water and nutrients at alkaline pH values of the fresh mortar mixture (pH 13 - 14), the biogranule structure does not deteriorate. Since the structure does not deteriorate, low solubility calcium salts other than biogranules can protect the spores from the mechanical stresses caused by hydration and increased volume during the hardening of the mortar. Therefore, the spores added to the mixture in the form of biogranules can last for a long time in cement composites [66], [68].

In the thesis study, the success criterion for the experiments performed on fresh mortar mixtures was determined as "The amount of bacteria in the form of maximum biogranules that can be used in bioconcrete corresponds to 0.50% or more of cement by weight with positive results in the literature". Considering that the maximum amount of bacteria in the form of biogranules that can be used in fresh mortar experiments was determined as 2.50%, it can be said that the part related to fresh mortar experiments was successfully completed without the need for any setting accelerator or super plasticizer.

4.3. Characteristics of Cured Biomortar Samples and the Maximum Usable Bacterial Dosage

In the thesis study, compression strength tests were applied to the hardened mortar samples at the end of 3, 7, 28 and 56 days. In this context, the compressive strength of the samples prepared according to the EN 196-1 standard was tested in sealed plastic bags after curing at room temperature for 3, 7, 28 and 56 days. Compressive strengths obtained on different days are presented in Figure 4.14. Samples were compared with reference (or plain control) sample, control (abiotic control) sample and statistical analysis among themselves in terms of compressive strength obtained at different times. According to these results, there is no significant difference between reference mortar and control mortar samples in terms of early age strength ($p = 0.05$) and 3-day compression strengths were obtained as 29 ± 4 MPa and 30 ± 2 MPa, respectively. These findings are in parallel with previous study [67]. When bacteria were added in the form of biogranules, it was found that adding bacteria up to a dose of 2.00% by weight of cement did not have a statistically significant effect on 3-day strength performance ($p = 0.05$). However, adding bacteria at a dose of 2.50% and 3.00% even in the form of biogranules caused a decrease of 26% in 3-day strength compared to the reference value (Figure 4.15). The main reason for this is thought to be related to the settlement problem of the concrete with low workability, because the 3-day strengths of the other samples with good settling did not show a statistically significant difference from the reference and control samples ($p = 0.05$). Although the 3-day strengths of the Bio-2.50% and Bio-3.00% samples were lower, at the end of 7 days, all biomortars reached a compression strength of around 40 MPa and did not differ statistically from each other ($p = 0.05$). Both the control and biomortar samples performed better than the reference mortar sample at the end of 28 days ($p = 0.05$). While the 28-day compressive strength value of the reference mortar sample was measured as 43 ± 3 MPa, both control and bio-mortar samples showed a 30% better performance and reached strengths around 56 MPa (Figure 4.15). The 56-day compression strength value of the reference mortar sample was measured as 52 ± 2 MPa. Except for the Bio-3.00% sample, all other mortar mixtures performed 15% to 25% better than the reference sample in terms of 56 days compressive strength (Figure 4.15). There was no statistically significant difference between the Reference and Bio- 3.00% ($p = 0.05$). In addition, it was determined that all bio-mortar samples, except Bio-3.00%, had

statistically similar strengths both with the control sample and among themselves (p = 0.05).

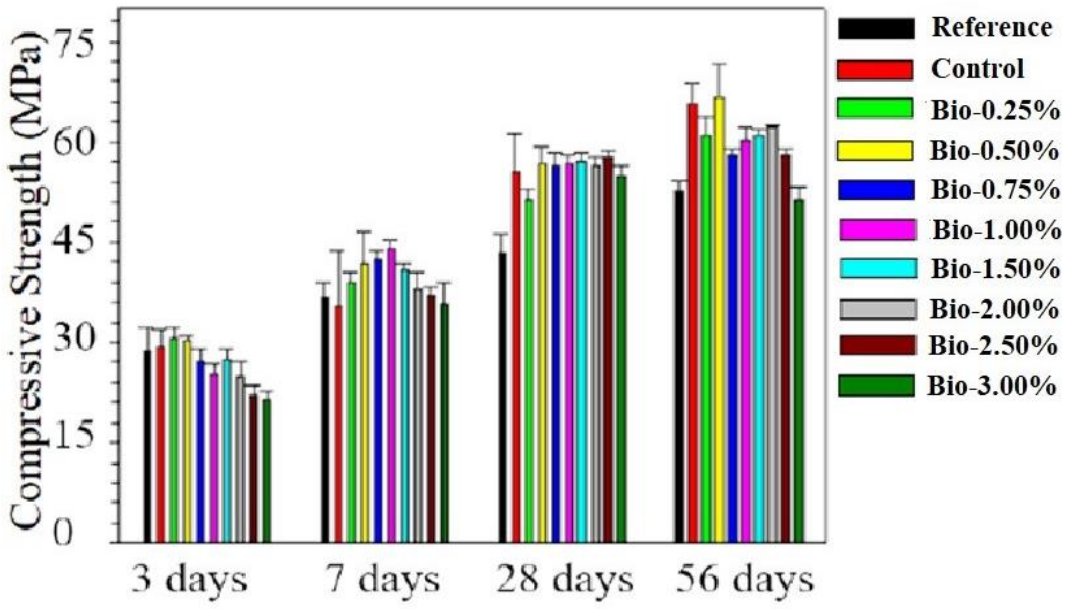

Figure 4. 15. Compressive strength of the tested mortar samples with different content at the end of 3, 7, 28 and 56 days. Mean values are presented in the graph, and error bars show the standard deviation value (n = 3).

The strength developments of the mortar samples based on time are important in terms of understanding whether the additives added to the mortar mixture have an effect on the hydration reactions that play a role in early age resistance. Therefore, the strength values of the mortar mixtures on the 3rd, 7th and 28th days were compared according to the highest strength values (56-day strength values) reached in the tests performed within the scope of the thesis study, and the strength developments of all mixtures were examined (Table 4.2). The strength development of Bio-2.50% and Bio-3.00% mixtures in the first 3 days was statistically significantly slower than both the reference sample and the control sample (p = 0.05). It was determined that the strength development of the Bio-2.00% sample in the first 3 days was slower than only the reference sample (p = 0.05). Thus, the compressive strength values of the Bio-2.50% and Bio-3.00% samples were also obtained weaker than the reference and control samples in the first three days. However, although the strength development in the first 3 days was slower than the reference sample, there was no problem with the strength in the Bio-2.00% sample (Table 4.2, Figure 4.15).
It was determined that the strength developments in the first 7 days and the first 28 days did not show a statistically significant difference in different mortar mixes (p = 0.05).

Table 4. 2. Strength development of mortar mixes in different time periods.

Mortar mixtures	Percentages of strength development in early age hydration process (%)			
	3rd Day	**7th Day**	**28th Day**	**56th Day**
R	55±8	70±6	85±6	100
C	48±4	57±7	91±9	100
Bio-0.25%	47±4	64±3	84±2	100
Bio-0.50%	46±5	62±2	90±10	100
Bio-0.75%	47±4	73±2	96±3	100
Bio-1.00%	42±5	73±4	97±3	100
Bio-1.50%	45±4	67±2	93±3	100
Bio-2.00%	42±3	61±4	91±2	100
Bio-2.50%	38±3	64±2	99±2	100
Bio-3.00%	41±1	70±8	104±4	100

The compression strength results of the control sample are consistent with the performance increase of 20% obtained in the study of the thesis co-advisor, in which he examined the effect of calcium nitrate and calcium formate amounts on the compression strength [67]. In a recent study, the compressive strength of a mortar containing 3.00% calcium nitrate by weight of cement is similar to that of a plain mortar mixture, on the other hand, the compressive strength of a mortar containing 3.00% calcium formate by weight of cement is 27% higher than that of plain mortar[133]. Therefore, in this thesis, it was concluded that the increase observed in compressive strength probably developed due to the calcium form and that the bacteria added in the form of biogranules did not have a significant effect on the compression strength. Similarly, it was reported that the addition of biogranules did not have a negative effect on mechanical properties [88]

The 3-day strengths of bio-mortars containing only Bio-2.50% and Bio-3.00% of the mixtures tested were weaker compared to the reference and control samples (Figure 4.15). In the same mixtures, a slowdown was observed in the development of early age strength only for the first 3 days (Table 4.2). In the strength development in the first 3 days, Bio-2.50% and Bio-3.00% as well as Bio-2.00% are slower than the reference sample. However, in the compressive strength obtained in the first 3 days, poor performance was

obtained only in Bio-2.50% and Bio 3.00% mixtures, which also had settlement problems. In the first 3 days of strength development, although all three samples perform 10% worse than the reference sample, the 3-day compressive strength is only low in Bio-2.50% and Bio 3.00% mixtures with settlement problem, due to the negative effect of biogranules on mortar hydratio rather, it shows that the high porosity formed in samples that do not settle well has a negative effect on the strength development and compression strength.

4.4. Bioconcrete Performances in Different Bacterial Doses

In order to observe the effect of biogranule addition on concrete, a bioconcrete mixture containing two different bacterial doses was also tested. One of the mixtures is BioC-0.25% (containing 0.25% bacteria in the form of biogranules by weight of cement) containing the lowest bacteria dose tested in fresh and hardened mortar experiments. The second mixture that is similar to the other bio-mortar samples and control samples in terms of all other properties (setting, flow, 7-, 28- and 56-days compression strength) except for settling problems and 3-day compression strength and which contain the maximum testable bacteria dose, is BioC-2.50% (containing bacteria in the form of biogranules at the rate of 2.50% by weight of cement).

During the thesis study, biogranules were produced in an amount that would enable the tests to be carried out on the bio-mortar samples and, when necessary, the casting of new bio-mortar samples. Pouring concrete samples containing the same dose of bacteria requires approximately 10 times more biogranules than mortar samples. For this reason, the biogranules produced during the thesis allowed the casting of concrete samples that will only be used in the 28 and 56-day strength tests. Since the continuity of granule production is disrupted due to the COVID-19 pandemic process, bio-concretes could not be tested in terms of 3 and 7-day compression strength and self-healing capacity.

4.4.1. Workability Properties of Bioconcrete Samples

The slump test on fresh concrete samples was carried out in accordance with EN 12350-2 standard. Using the obtained slump values, the consistency and workability of bio-concrete materials were compared with reference and control samples, taking into account the minimum 50 ± 25 mm and maximum 80 ± 25 mm slump values defined as limit values

in the EN 12350-2 standard. The slump values of the tested concrete mixtures are given in Table 4.3.

Table 4. 3. Slump values of different concrete mixes.

Mixtures	Slump Value (mm)
Reference	80
Control	105
BioC-0.25%	147
BioC-2.50%	40

According to the data obtained, it can be said that the reference sample is within the limit values in the EN 12350-2 standard. Although the control sample was determined to be more processable, it was determined that the slump values of the reference sample and the control sample were similar within the standard deviations given in the EN 12350-2 standard. It was determined that the processability of the BioC-0.25% mixture was better than the reference and control samples, but the processability of the BioC-2.50% sample was worse than the reference and control samples. Nevertheless, since the BioC-2.50% sample had a margin of 50 ± 25 mm, the minimum slump value in the EN 12350-2 sample, it was identified as an acceptable mixture with low machinability.

The workability results obtained in the fresh concrete test for the same mixtures and the workability results obtained in the fresh mortar tests confirm each other. This has once again revealed that, especially in small-scale studies, mortar samples can be tested as representative concrete samples.

4.4.2. Compressive Strength Performance of Hardened Concrete Specimens

The effect of the amount of bacteria on the hardened concrete properties was evaluated on the compressive strength of 28 and 56 days due to the limited amount of biogranules. The strength results obtained are presented in Table 4.4 and Figure 4.16.

Table 4. 4. 28- and 56-days compressive strength of the poured concrete samples.

Mixtures	**28 days compressive strength (MPa)**	**56 days compressive strength (MPa)**
Reference	35±2	37±2
Control	34±2	37±4
BioC-%0.25	33±2	35±3
BioC-%2.50	32±3	35±5

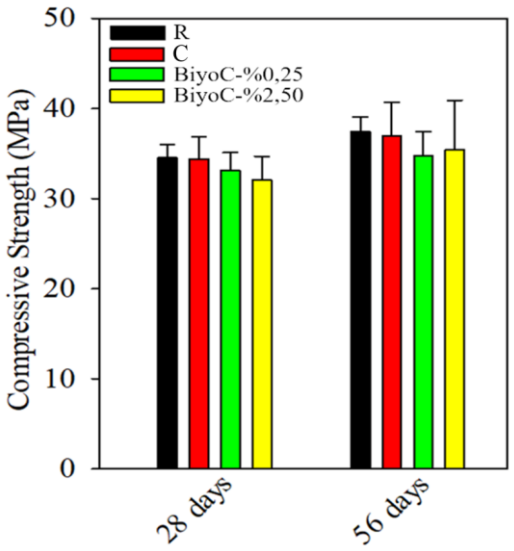

Figure 4. 16. Compressive strength of concrete samples. Mean values are given, error bars indicate standard deviation (n = 3).

Accordingly, it can be seen that the prepared reference mixture reaches the targeted pressure value as C25 / 30 at the end of 28 days (Figure 4.16). It was determined that the compression values of the control and bio-concrete samples were not statistically significantly different from the reference sample (p = 0.05). Likewise, all bio-concrete samples showed similar strength performances after 56 days. These results are different from the results obtained with the bio-mortar samples in one way or another. In hardened mortar samples, 28- and 56-days compression strengths of control, Bio-0.25%, and Bio-2.50% samples are similar among themselves, while the compressive strengths of all three mixtures have shown approximately 25% better performance in terms of strength at 28 days and 15% to 19% better performance in terms of strength at 56 days compared to the reference mixture. However, similar performance improvements could not be obtained in tests performed with concrete samples. It has been determined that the main reason for

the increase in compressive strength in mortar mixtures is calcium formate consisting of very fine particles. The difference observed between the mortar and concrete samples could not be explained in detail, since it remains unknown in what sense the calcium formate has a positive effect on the compressive strength and further analysis is outside the scope of the thesis. However, two basic hypotheses that may have caused the increase in compressive strength seen in mortar samples to not be seen in concrete samples are emphasized. The first of these is that the reduced surface areas due to the increased aggregate sizes compared to the mortar sample and the decrease in the amount of calcium formate particles falling into the interface transition zone (interfacial transition zone) may have eliminated the filling effect that the thin calcium formate particles may have created in the mortar sample. The second is that the water cement ratio used in mortar mixtures as 0.5 is used as 0.6 in concrete samples, so more calcium formate dissolves in water during the mixing and the filling effect of fine particles disappears. Thus, there are findings indicating that when 2.00% of calcium formate by weight of cement is dissolved in water and added to the mortar mixture, there is a positive effect on compressive strength [88].

Bacterial additions to the mortar are reported to cause an increment in early age due to the microbial calcium carbonate precipitation in many microorganisms added researches [102], [103], [134]. Nevertheless, the pH-value of biogranules was about 10 and the nutrients at the actual cementitious materials pH were neither germinated, nor used, making MICP unlikely during hydration. There is therefore no substantial improvement via the integration of bacteria in this thesis. The compatibility of biogranulations was verified by tests performed with concrete samples because similar compressive strengths was obtained in different mixes. Using a higher water-cement ratio in concrete tests could develop the workability of BioC-2.50%, resulting in all specimens reaching similar strength values in the same curing period. As a result, if a bacteria dose in the form of biogranules of more than 2.50% w/w cement is required, superplasticizers may be taken into account.

One of the major goals of this thesis was to show the production process of non-axenic nitrate-reducing biogranules designed for self-healing concrete applications and to evaluate their compatibility with the cementitious matrix at various application doses. Because of their limited interruption with the cementitious matrix, the produced

biogranules were found to be compatible with both mortar and concrete up to a dose of 3.60% w/w cement. The maximum acceptable dose allowed for the integration of bacteria up to 2.50% w/w cement without affecting setting time, workability, or compressive strength development. It is convincing to further examine cost-effective production of healing agents in higher proportions due to such yields are achieved under minimal nutrients conditions. More studies should be carried out to optimize the biogranular nitrate-reduction content based on the differences in the self-healing properties of biomortars at various doses of the biogranule.

4.5. The Effect of Bacteria Dose on the Self-Healing Capacity of Biomortars

The cracked bio-mortar samples were placed in containers so that they were completely submerged in tap water and the weekly change of the crack width was monitored under the microscope. Crack healing percentages of the samples were calculated weekly according to the measurements under the microscope. In order to compare autogenous and autonomous healings, reference and abiotic control samples were also examined along with biogranulated samples.

4.5.1. Reference Sample and Autogenous Crack Healing Performance

Weekly improvement graphs of reference samples are presented in Figure 4.17. Crack widths obtained in reference samples varied between 65 μm and 330 μm (Figure 4.17). In reference samples, the highest crack healing performance in the first week was obtained in cracks varying between 59 ± 5% and 260 μm and 330 μm ($p = 0.05$) (Figure 4.17-a). In addition, crack widths between 105 μm and 235 μm showed similar improvement, while the worst healing performance was obtained in cracks smaller than 100 μm ($p = 0.05$). At the end of the second week, the percentage of closure in cracks varying between 160 μm and 330 μm increased up to 70% and the crack closure percentages for the same interval were recorded as 72% and 76% at the end of the third and fourth weeks, respectively (Figure 4.17-b, c, d). In this process, a lower percentage of closure was obtained in cracks varying between 65 and 135 μm compared to crack widths of 160-330 μm ($p = 0.05$). A representative visual of autogenously healing cracks in the reference sample is given in Figure 4.18.

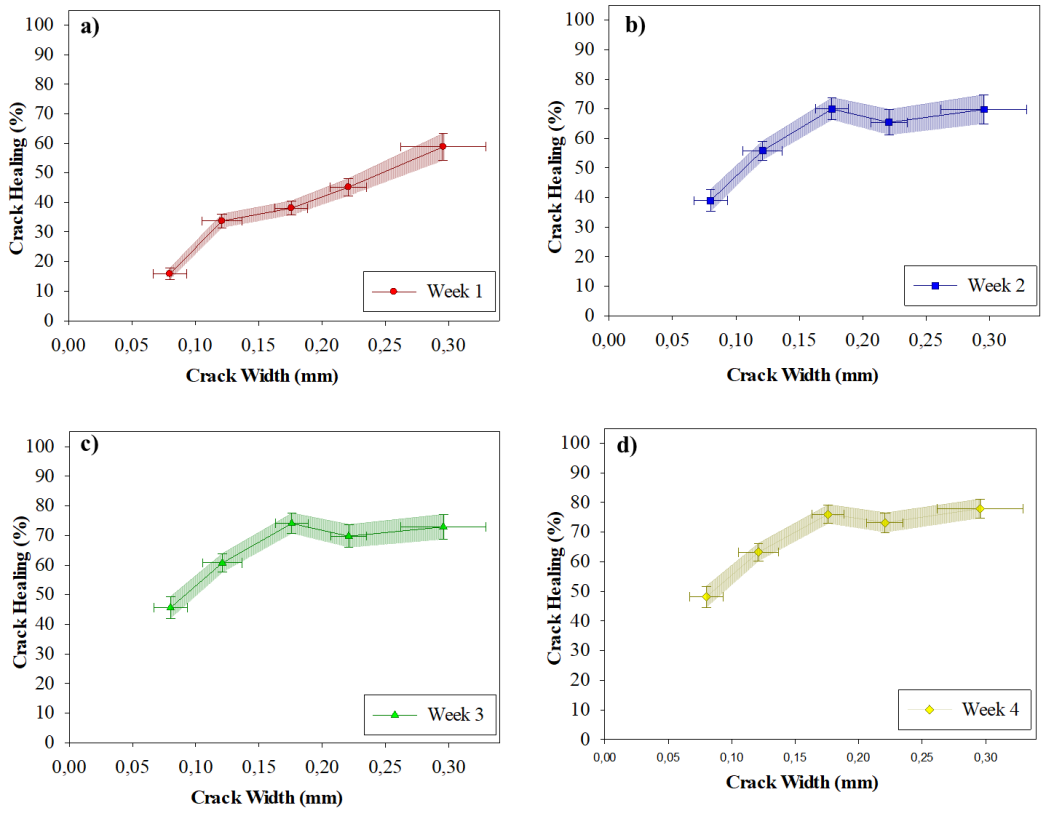

Figure 4. 17. Underwater crack healing performances of reference samples (a) after 7 days; (b) after 14 days; (c) after 21 days; (d) after 28 days. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

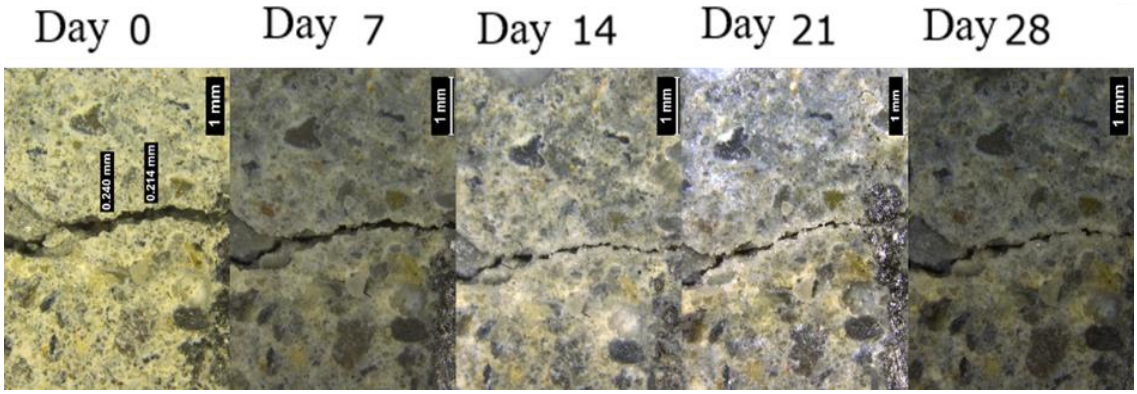

Figure 4. 18. Micrographs showing autogenous healing during the curing period.

Within the scope of this thesis, the samples with a crack healing efficiency of 90% and above were determined as the self-healing cement composite and the largest crack healing 90% and above as the self-healing capacity of the self-healing cement composite. Therefore, although the reference sample showed an autogenous closure in cracks, it does

not fall into the self-healing cement composite class defined in this study as the percentage of closure is limited at 78%.

In Figure 4.19, the crack healing performance of reference samples over 4 weeks is given in a single graph. As seen in Figure 4.19, there was no statistically significant change in the percentage of cracks closing after the second week ($p = 0.05$). This shows that autogenous healing is completed in the first two weeks. Therefore, it is thought that the autogenous healing in the reference samples mainly occurs when the cement particles whose hydration is not completed come into contact with water after crack formation and the hydration reactions continue.

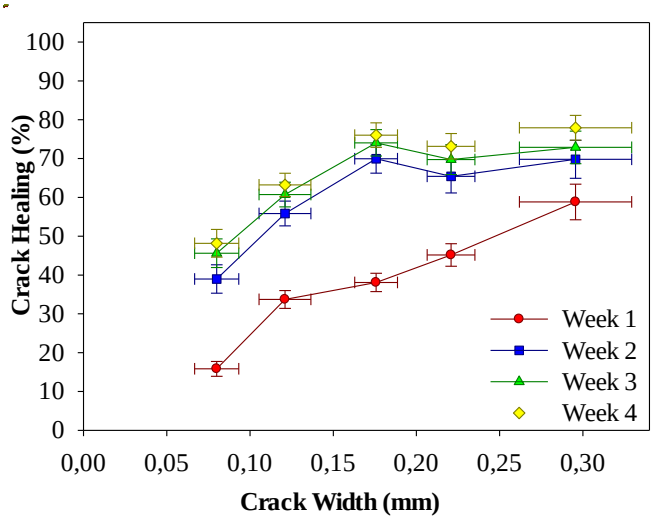

Figure 4. 19. Underwater crack healing performances of reference samples (a) after 7 days; (b) after 14 days; (c) after 21 days; (d) after 28 days. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

Since there is no crack healing of 90% or more in the reference samples, the crack widths that heal the most in different curing times and the highest crack healing percentages seen in these cracks were determined and presented in Table 4.5.

Table 4. 5. Crack widths and healing percentages of reference samples showing the greatest improvement at different times[1].

Duration	Best healed crack widths (μm)	Crack healing ratio (%)
Week 1	295±34	59±5
Week 2	160 – 330	68±5
Week 3	160 – 330	72±4
Week 4	160 – 330	76±3

[1]Since the healing performances of the cracks between 160 - 330 μm after the second week were not statistically different (p = 0.05), the healing percentages of the crack widths in the given range were averaged.

It is noteworthy that crack closure occurs primarily in wide cracks in reference samples. In the literature, crack intervals given for 28-day autogenous healing under submerged conditions vary between 30 μm and 250 μm [26], [28], [29], [95], [135]. This is due to the dependence of autogenous healing on parameters such as the amount of unhydrated cement particles, which can vary from crack to crack, and carbon dioxide holding capacity, which varies from sample to sample. Therefore, in the literature, autogenous healing is defined as a type of healing that cannot be predicted, and this situation is shown as one of the main reasons for the investigation of autonomous healing types [28]. It can be said that the higher healing efficiencies observed in this study where crack width is high are also due to the effect of unpredictable variables in the structure of reference samples that can only autogenously heal.

4.5.2. Control Sample and Autogenous Crack Healing Performance

For the autogenous healing assessment, abiotic control samples containing basic mortar materials and nutrients (5.00% calcium formate and 2.00% calcium nitrate by weight of cement) were used in addition to the reference samples. Weekly crack healing graphs of control samples are shown in Figure 4.20.

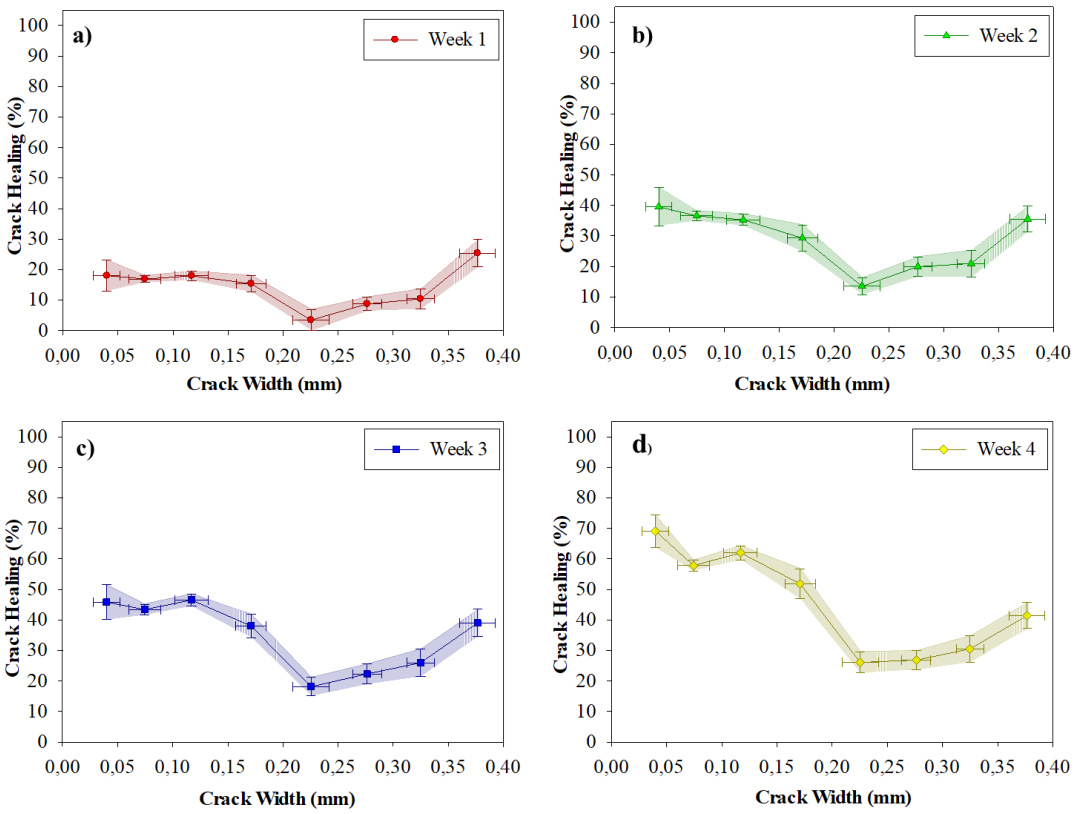

Figure 4. 20. Underwater crack healing performances of ontrol samples (a) after 7 days; (b) after 14 days; (c) after 21 days; (d) after 28 days. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

The crack widths obtained in the control samples varied between 30 µm and 390 µm (Figure 4.20). In the control samples, the highest crack healing performances were observed in cracks varying between 30 µm and 185 µm and 360 µm and 390 µm with approximately 20% closure in the first week ($p = 0.05$) (Figure 4.20-a). The same crack gaps were better than cracks between 210-340 µm with their closing performance of about 35% in the second week and they showed a similar healing performance among themselves ($p = 0.05$) (Figure 4.20-b). At the end of three weeks, approximately 40% improvement was observed in crack widths up to 185 µm and crack widths varying between 360 µm and 390 µm (Figure 4.20-c). At the end of four weeks, the highest crack closure rate was obtained in cracks smaller than 50 µm with 69 ± 5% (Figure 4.20-d). A representative visual of autogenously healing cracks in the control samples is given in Figure 4.21.

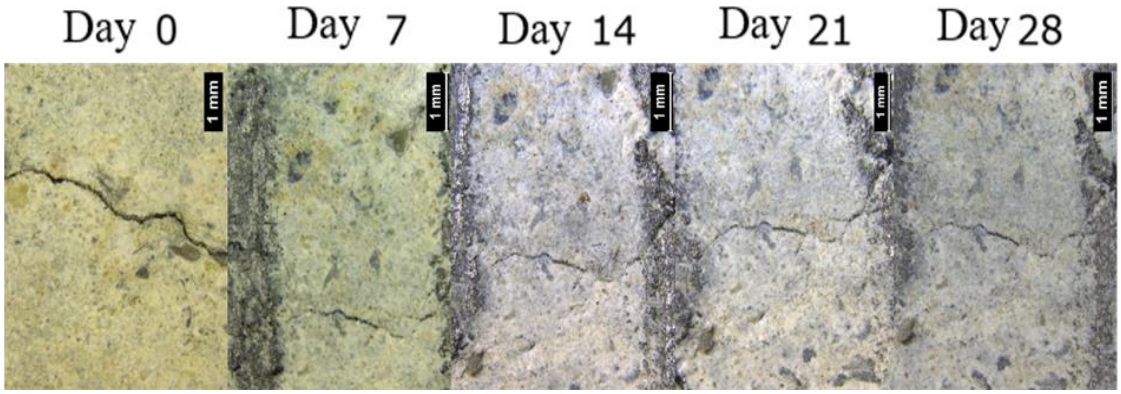

Figure 4. 21. Micrographs showing autogenous healing in control samples.

This mixture is not included in the self-healing cement composites class, since a crack healing rate of 90% is not observed in the control samples as well as in the reference samples. As in the reference samples, a consistent trend was not observed in the healing rates and closure rates of the cracks in the control samples (Figure 4.20). However, at the end of four weeks, it was determined that cracks smaller than 50 µm had the highest healing performance, cracks between 50 and 185 µm a little lower, and cracks larger than 185 µm had the lowest healing performance (p = 0.05) (Figure 4.22).

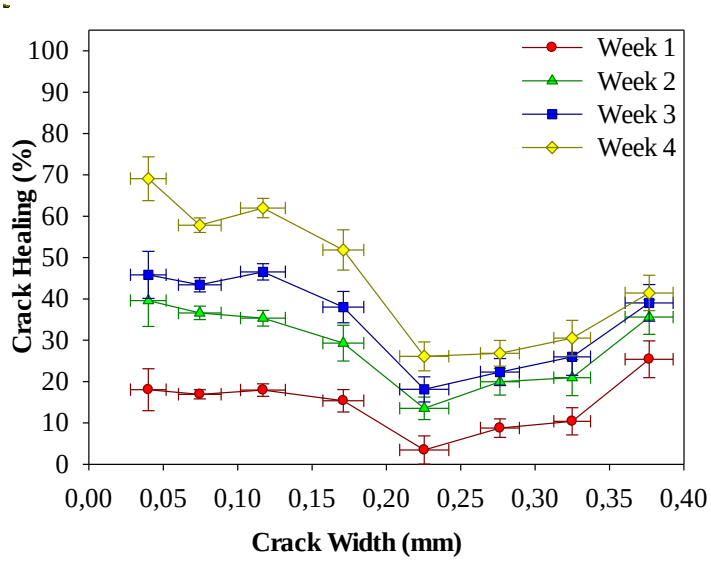

Figure 4. 22. Weekly change of crack healing performance in autogenous healing in control sample. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

Similar to the reference samples, there was no significant change in the percentage of closure in cracks larger than 200 µm after the second week in the control samples (p = 0.05) (Figure 4.22). The fact that the control samples also showed a difficult to predict

healing performance like the reference samples indicates that the nutrients added to the mortar did not have a positive effect on the crack healing performance. It can even be said that the control samples performed below the reference at the maximum crack closure percentages obtained at different crack widths (Table 4.5, Table 4.6).

Since there was no crack healing of 90% or more during the curing period in the control samples, the crack widths that healed the most in different curing times and the highest crack healing percentages observed in these cracks were determined and presented in Table 4.6.

Table 4. 6. Crack width and healing percentages of the control sample showing the highest healing at different duration.

Duration	Best healed crack widths (µm)	Crack healing ratio (%)
Week 1	30 – 185 ve 360 – 390	19±5
Week 2	30 – 135 ve 360 – 390	36±5
Week 3	30 – 135 ve 360 – 390	43±5
Week 4	40±10	69±5

[1]Since the healing performances of crack widths between 30 - 185 µm and 360 - 390 µm in the first three weeks are not statistically different (p = 0.05), the average of the healing percentages of the given crack widths is presented as the crack healing ratio.

4.5.3. Crack Healing Performance in Bio-0.25% Samples

Weekly crack healing graphs of samples containing 0.25% biogranule form of bacteria by weight of cement are shown in Figure 4.23. The crack widths obtained in the Bio-0.25% samples varied between 40 µm and 830 µm. In the first week, some cracks were covered by 100%, while some cracks closed around 80% (Figure 4.23). Although the crack healing ratio increased over 90% in many crack intervals at the end of the first week, the self-healing capacity was not determined for the first week due to the performance differences close to 20% in different crack intervals (Figure 4.23-a). It was determined that the highest crack width that healed over 90% at the end of the first week was 550 ± 30 µm. From the crack widths observed at the end of the second week, it was noted that there was 90% and more closure in all crack widths, except for crack widths varying between 615–635 µm (Figure 4.23-b). It was determined that all crack widths

observed at the end of three weeks showed healing over 90% and there was no significant change in terms of crack healing performance between the third and fourth weeks (p = 0.05) (Figure 4.23-c, d). A representative visual of the widest range of microbial healing cracks in Bio-0.25% samples is given in Figure 4.24.

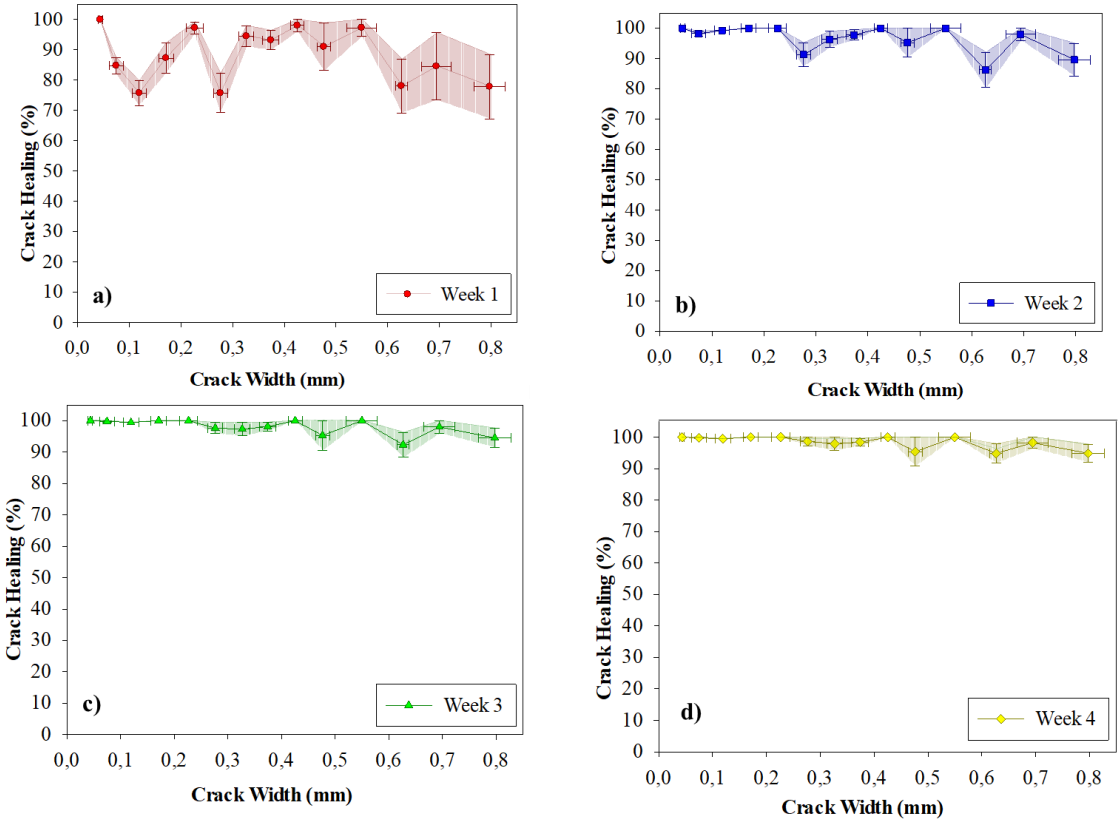

Figure 4. 23.Under water crack healing performances of Bio-0.25% samples (a) after 7 days; (b) after 14 days; (c) after 21 days; (d) after 28 days. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

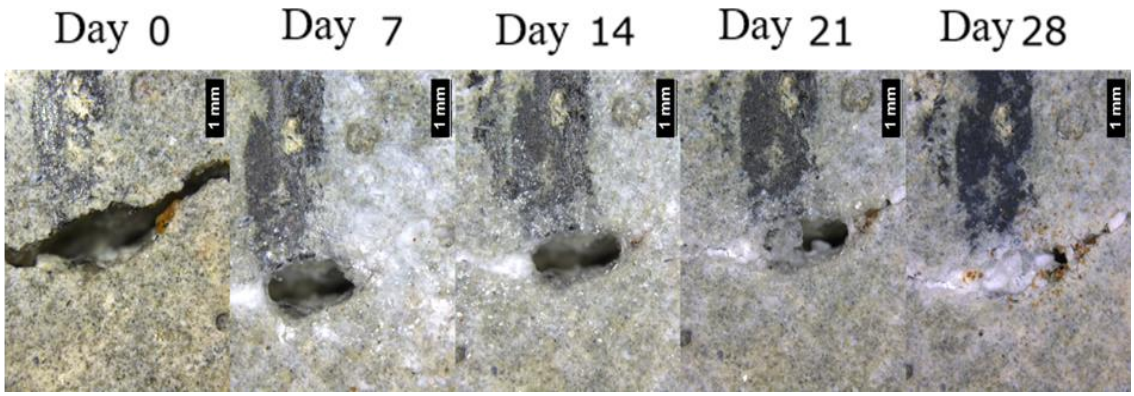

Figure 4. 24. Micrographs showing microbial healing in the Bio-0.25% sample.

Figure 4.25 shows the 4-week crack healing performance and determined self-healing capacity obtained in Bio-0.25% samples.

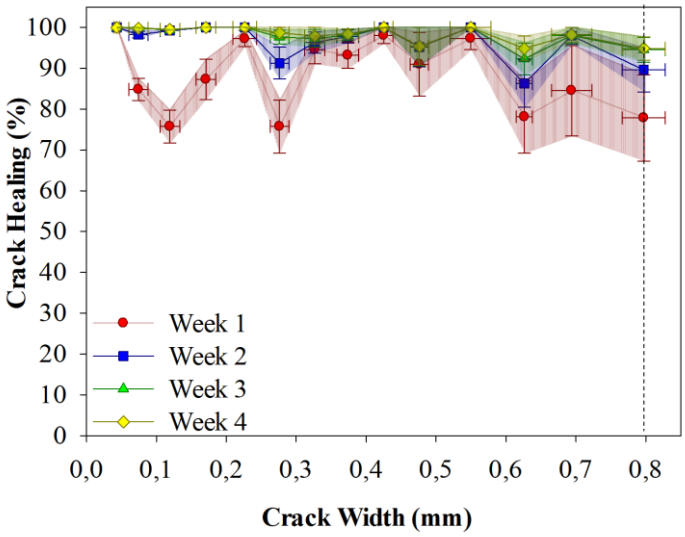

Figure 4. 25. Weekly change of microbial healing performance and determined self-healing capacity for Bio-0.25% samples. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

According to the results obtained, it can be said that the Bio-0.25% samples are a self-healing cement composite. The self-healing limit of the cement composite, which repairs itself with microbial means, was determined as 800 ± 30 µm and the healing period as 3 weeks. Crack widths, self-healing capacities and the highest crack healing percentages seen in different curing times are presented in Table 4.7.

Table 4. 7. Healing capacities and healing percentages of Bio-0.25% in different durations.

Duration	**Best healed crack widths (µm)**	**Crack healing ratio[1] (%)**
Week 1	<100, 155–245, 310–580, 660–720	92±7
Week 2	<580	95±5
Week 3	<830	97±3
Week 4	<830	98±2

[1] Due to the performance differences close to 20% in different crack intervals, the self-healing capacity could not be determined for the first week, and the average of the

healing percentages obtained in the given crack width ranges was presented as the crack healing ratio.

4.5.4. Crack Healing Performance in Bio-0.50% Samples

Weekly crack healing graphs of samples containing bacteria in the form of biogranules at 0.50% by weight of cement are shown in Figure 4.26.

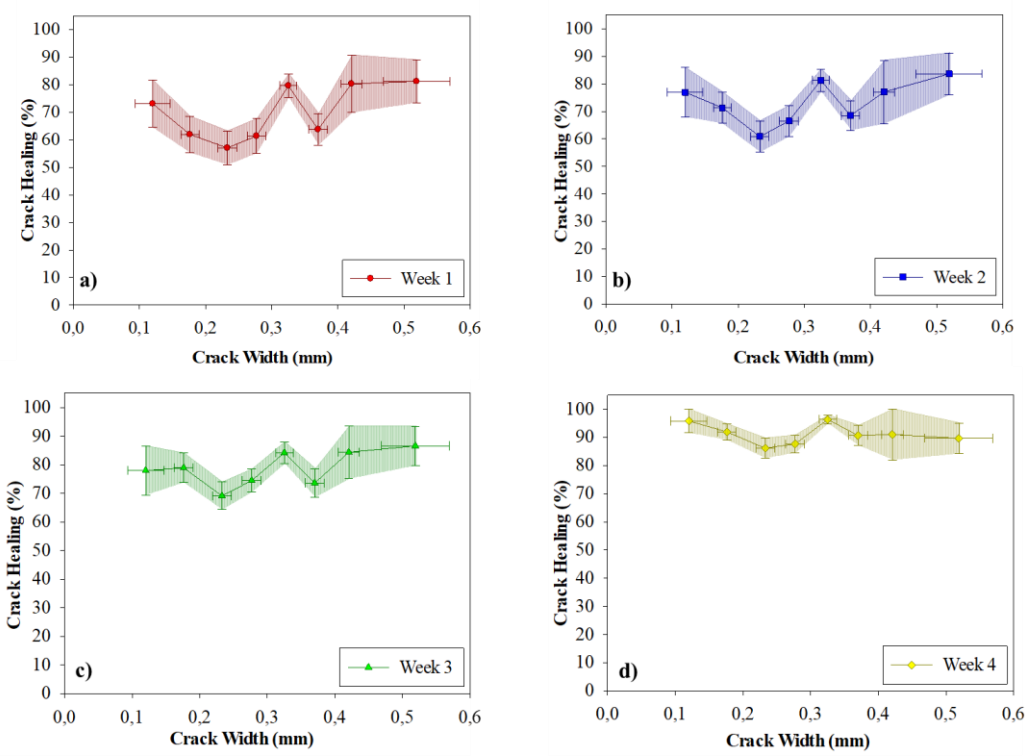

Figure 4. 26. Under water crack healing performances of Bio-0.50% samples (a) after 7 days; (b) at the end of the 14th day; (c) after 21 days; (d) after 28 days. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

The crack widths obtained in the Bio-0.50% samples varied between 100 µm and 570 µm. In the Bio-0.50% samples, healing performances varying between 57% and 81% were obtained in the first week, being similar in all observed crack widths ($p = 0.05$) (Figure 4.26-a). At the end of the second week, the healing ratios in all crack widths showed a similar increase and it was determined that the two-week crack healing performances varied between 61% and 83% (Figure 4.26-b). At the end of the third week, crack healing performance varying between 70% and 85% was achieved in all observed crack widths, while at the end of the fourth week, a crack closure percentage of 90% and

above was obtained in all crack widths except for crack widths varying between 220 and 290 µm (Figure 4.26-c, d). At crack widths below 90% (220-290 µm), the crack closure percentage was recorded as 87 ± 3%, and it was determined that this performance was not statistically different from the healing performances obtained in other crack widths (p = 0.05). A representative visual of the widest range of microbial healing cracks in Bio-0.50% samples is given in Figure 4.27.

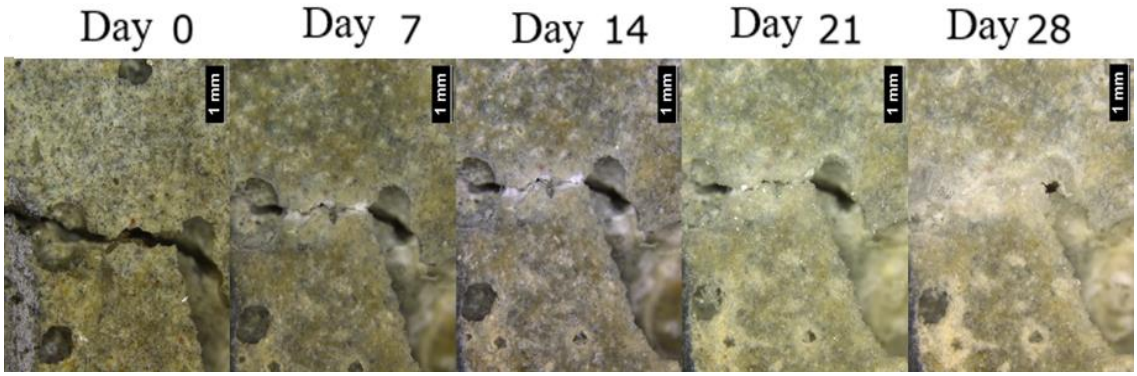

Figure 4. 27. Micrographs showing microbial healing in the Bio-0.50% sample.

Figure 4.28 shows the 4-week crack healing performance and self-healing capacity obtained in Bio-0.50% samples.

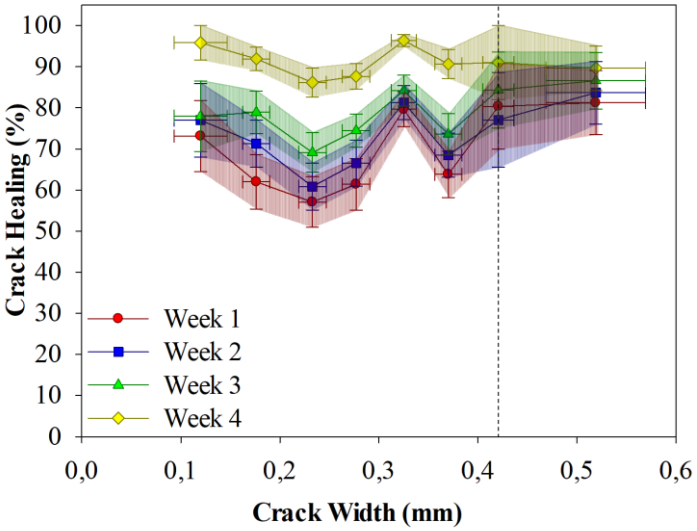

Figure 4. 28. Weekly change of microbial healing performance and determined self-healing capacity for Bio-0.50% samples. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

According to Figure 4.28, crack healing performances vary directly with the curing time. This situation is in line with the findings obtained in the previous studies of cement composites that repair themselves with microbial means containing similar doses of bacteria [95]. While the crack healing performance obtained in the first week shows a slower increase in the second and third weeks, it is seen that the increase with time shows parallelism with different crack widths (Figure 4.28). In the fourth week, it can be said that the healing rate of the cracks is higher than the other weeks (Figure 4.28). Considering that autogenous healing is dominant in the first two weeks, it can be said that the period in which the dominance of microbial healing is increased is the fourth week. The self-healing limit of the cement composite, which repairs itself with microbial means, was determined as 520 ± 50 μm after four weeks (Figure 4.28). Since there was no crack healing of 90% or more in the first three weeks in the Bio-0.50% samples, the crack widths that healed the most in different curing times and the highest crack healing percentages observed in these cracks were determined and presented in Table 4.8.

Table 4. 8. Crack widths and healing percentages of Bio-0.50% showing the highest healing in different durations.

Duration	**Best healed crack widths (μm)**	**Crack healing ratio[1] (%)**
Week 1	<570	70±11
Week 2	<570	73±10
Week 3	<570	79±8
Week 4	<570	91±5

[1]As the crack healing ratio, the average of the healing percentages obtained in the given crack width ranges is presented.

In the Bio-0.50% samples, crack healing of 90% or more could not be obtained for any crack width in the first three weeks. Therefore, it can be said that the Bio-0.50% sample is not suitable for self-healing bio-concrete applications that are expected to heal cracks before 4 weeks.

4.5.5. Crack Healing Performance in Bio-0.75% Samples

Weekly crack healing graphs of samples containing bacteria in the form of biogranules at a rate of 0.75% by weight of cement are shown in Figure 4.29.

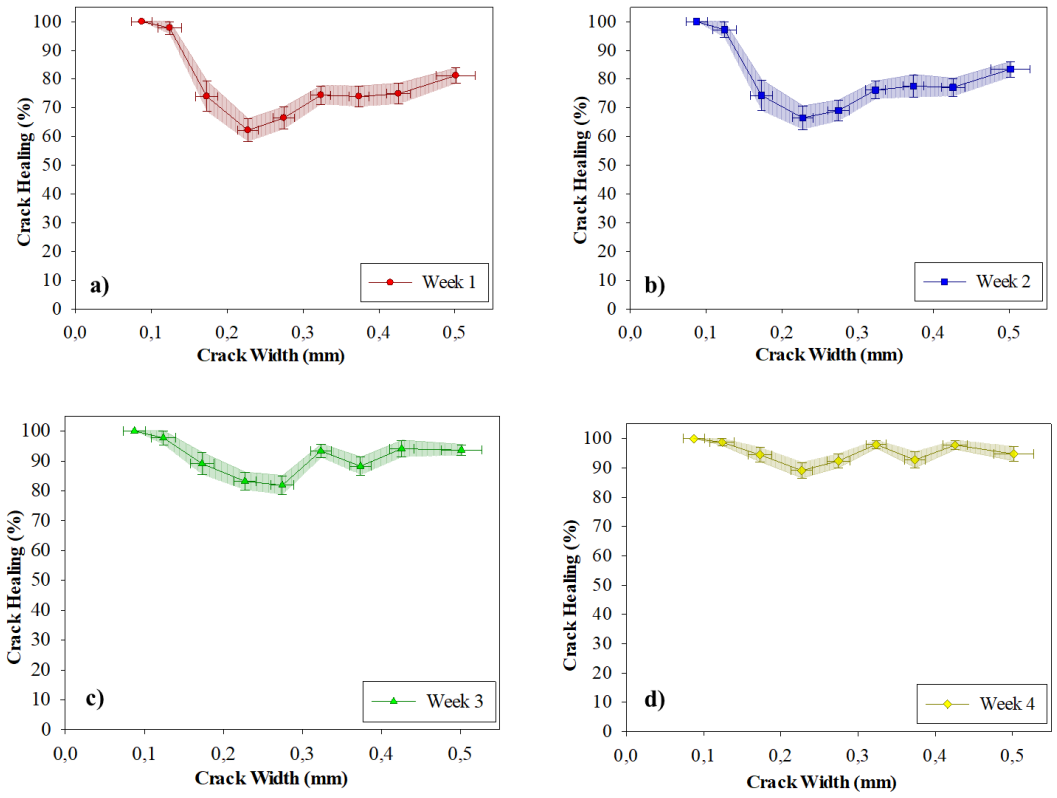

Figure 4. 29. Under water crack healing performances of Bio-0.75% samples (a) after 7 days; (b) at the end of the 14th day; (c) after 21 days; (d) after 28 days. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

The crack widths obtained in the Bio-0.75% samples varied between 75 µm and 525 µm (Figure 4.29). In the Bio-0.75% samples, the highest crack healing performance was observed at the crack widths less than 140 µm at the end of the first week and it was determined that these crack widths were completely healed (Figure 4.29-a). At the end of the second week, no statistically significant improvement was observed in all observed crack widths compared to the first week ($p = 0.05$). At the end of the third week, it was observed that all crack widths were closed more than 90% except the crack widths between 215 µm and 290 µm (Figure 4.29-c). At the end of the first three weeks, there is an 80% closure at crack widths between 215 µm and 290 µm. At the end of the fourth week, 90% and more closure was obtained in all crack widths observed (Figure 4.29-d).

A representative visual of the widest range of microbial healing cracks in bio-0.75% samples is given in Figure 4.30.

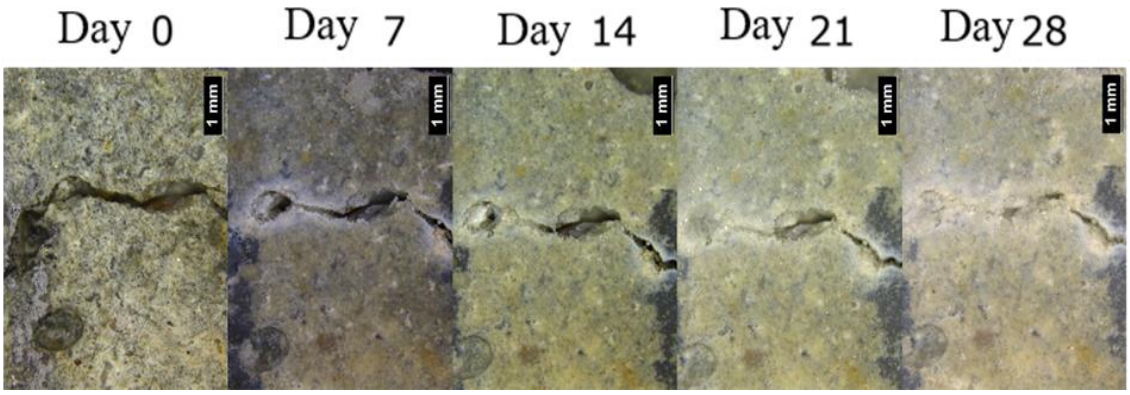

Figure 4. 30. Micrographs showing microbial healing in the Bio-0.75% sample.

Figure 4.31 shows the 4-week crack healing performance and determined self-healing capacity obtained in Bio-0.75% samples. Bio-0.75% is included in the self-healing cement composite class within the scope of this thesis, as crack healing of 90% or more is achieved at all cracks widths observed at the end of the curing period.

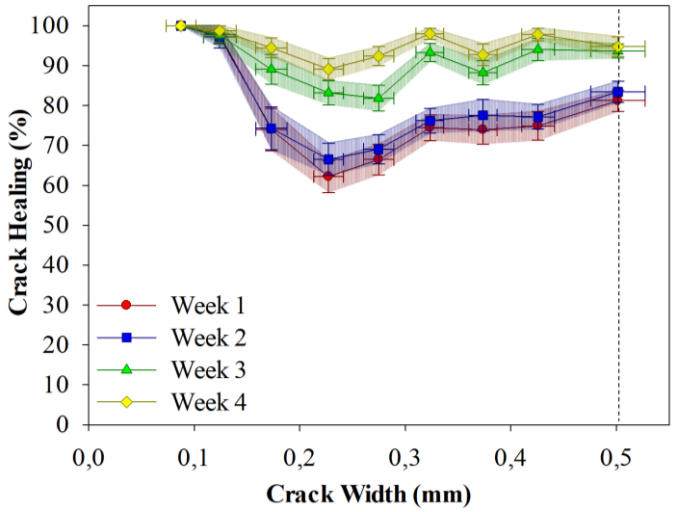

Figure 4. 31. Weekly change of microbial healing performance and determined self-healing capacity for Bio-0.75% samples. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

Similar to Bio-0.50%, the performances of the first two weeks and the performances afterwards in the Bio-0.75% samples are separated from each other. Bio-0.75% samples, as in other bio-mortar samples, have improved their healing performance to a certain level

in the first two weeks where autogenous healing is dominant; however, unlike autogenous healing, crack closure percentages increased in the third and fourth weeks (Figure 4.31). The absence or very weakness of this ongoing increase in reference and control samples indicates a microbial healing in bio-mortar samples. The self-healing limit of the cement composite, which repairs itself with microbial means, was determined as 500 ± 25 μm after four weeks (Figure 4.31). Since a statistically significant improvement of 90% and above from the first week in Bio-0.75% samples, the self-healing capacity was determined for Bio-0.75% at different curing times and is presented in Table 4.9.

Table 4. 9. Self-healing capacity and healing percentages of Bio-0.75% in different durations.

Duration	**Best healed crack widths (μm)**	**Crack healing ratio[1] (%)**
Week 1	<140	99±2
Week 2	<140	98±3
Week 3	<190 ve 310–525	93±5
Week 4	<525	95±4

[1]As the crack healing ratio, the average of the healing percentages obtained in the given crack width ranges is presented.

4.5.6. Crack Healing Performance in Bio-1.00% Samples

Weekly crack healing graphs of samples containing bacteria in the form of biogranules at the rate of 1.00% by weight of cement are shown in Figure 4.32. The crack widths obtained in the Bio-1.00% samples varied between 35 μm and 670 μm. At the end of the first week at bio- 1.00%, the highest crack healing performance was observed at crack widths smaller than 190 μm and it was determined that these crack widths improved by approximately 90% (Figure 4.32-a). At the end of the second week, while there was no significant increase in the closing rates of cracks smaller than 190 μm, it was found that the closing rate increased in wider cracks (Figure 4.32-b). Crack closure percentages increased in the third week and the maximum crack width which showed improvement over 90% increased to 280 μm (Figure 4.32-c). The maximum crack width that healed after four weeks was recorded as 330 μm (Figure 4.32-d). It has been observed that the

percentage of healing in larger cracks decreases in parallel with the increase in crack width. A representative visual of the widest crack range that heals bio-microbially at 1.00% is given in Figure 4.33.

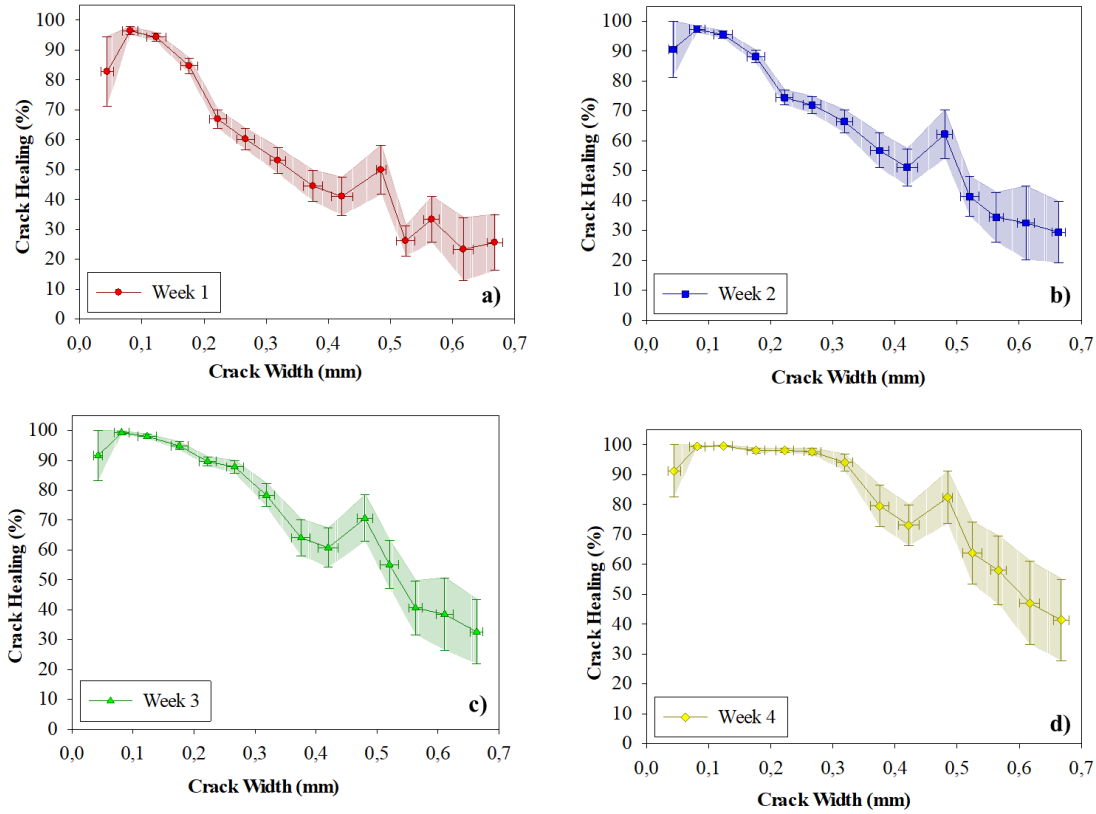

Figure 4. 32. Under water crack healing performances of Bio-1.00% samples (a) after 7 days; (b) at the end of the 14th day; (c) after 21 days; (d) after 28 days. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

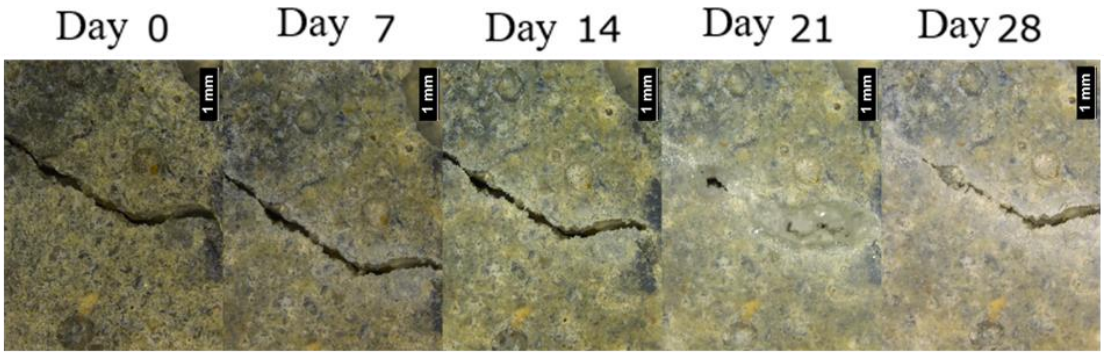

Figure 4. 33. Micrographs showing microbial healing in Bio-1.00% sample.

In Figure 4.34, 4-week crack healing performance and self-healing capacity obtained in Bio-1.00% samples are given. Bio-1.00% was included in the self-healing cement composite class within the scope of this thesis, as over 90% improvement was achieved in crack widths up to 330 µm at the end of the curing period.

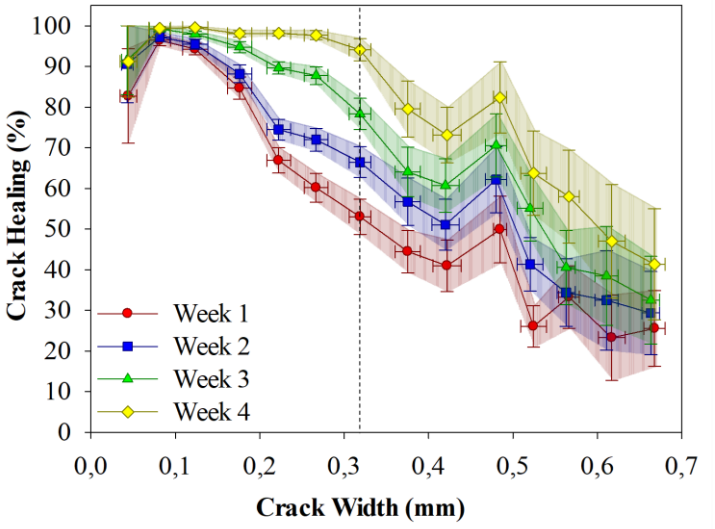

Figure 4. 34. Weekly change of microbial healing performance and determined self-healing capacity for Bio-1.00% samples. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

Similar to Bio-0.50% and Bio-0.75%, the first two weeks performances and the performances afterwards are separated from each other in Bio-1.00%. At bio-1.00%, as in other bio-mortar samples, healing performances increased to a certain level in the first two weeks where autogenous healing was dominant, but unlike autogenous healing, crack closing percentages were also increased in the third and fourth weeks (Figure 4.34). The self-healing limit of the cement composite, which repairs itself with microbial means, was determined as 320 ± 10 µm after four weeks (Figure 4.34). As in Bio-1.00%, as in Bio-0.75, a statistically significant healing of 90% and above from the first week was obtained, the self-healing capacity was determined for Bio-1.00% at different curing times and it is presented in Table 4.10.

Table 4. 10. Self-healing capacity and healing percentages of the Bio-1.00% sample at different durations.

Duration	Best healed crack widths (µm)	Crack healing ratio[1] (%)
Week 1	<190	91±8
Week 2	<190	93±5
Week 3	<280	94±5
Week 4	<330	96±5

[1] As the crack healing ratio, the average of the healing percentages obtained in the given crack width ranges is presented.

4.5.7. Crack Healing Performance in Bio-1.25% Samples

Weekly crack healing graphs of samples containing bacteria in the form of biogranules at 1.25% by weight of cement are shown in Figure 4.35.

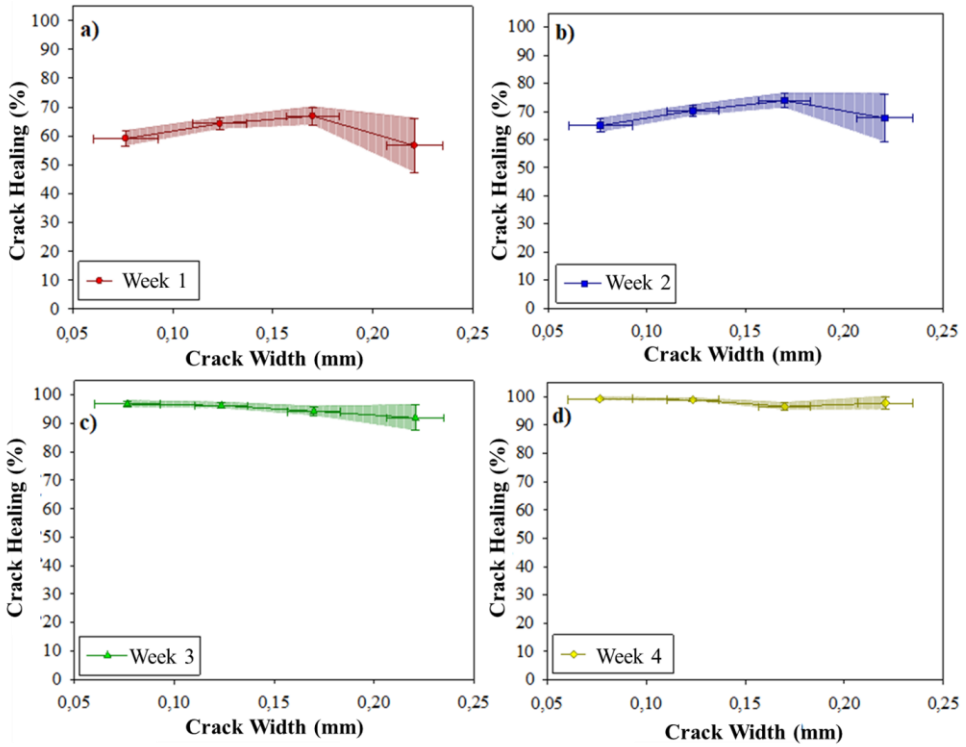

Figure 4. 35. Under water crack healing performances of Bio-1.25% samples (a) after 7 days; (b) at the end of the 14th day; (c) after 21 days; (d) after 28 days. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

The crack widths obtained in the Bio-1.25% samples varied between 35 μm and 235 μm. In the Bio-1.25% samples, healing performances varying between 56-68% were obtained in the first week, being similar at all crack widths (p = 0.05) (Figure 4.35-a). At the end of the second week, the healing rates in all crack widths showed a similar increase and it was determined that the crack healing performance for two weeks varied between 65% and 75% (Figure 4.35-b). At the end of the third week, over 90% of crack healing performance was obtained in all observed crack widths and minimal changes were observed in crack closure percentages in the fourth week (Figure 4.35-c, d). A representative visual of the widest range of microbially healing cracks in Bio-1.25% samples is given in Figure 4.36.

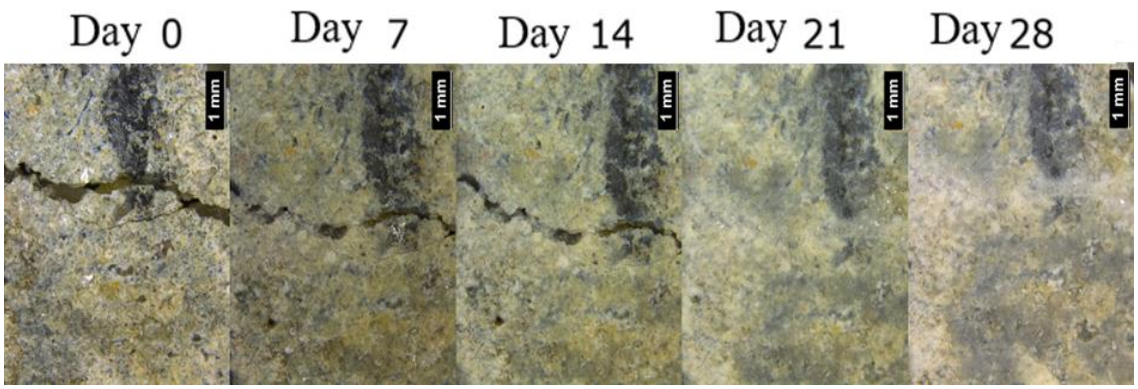

Figure 4. 36.Micrographs showing microbial healing in Bio-1.25% sample.

In Figure 4.37, 4-week crack healing performance and self-healing capacity obtained in Bio-1.25% samples are given. Bio-1.25% was included in the self-healing cement composite class within the scope of this thesis study, as over 90% improvement was achieved in crack widths up to 235 μm at the end of the curing period. As in other tested bio-mortar samples, the first two weeks performances and the performances afterwards are separated from each other in Bio-1.25% (Figure 4.37). After the second week in which autogenous healing is estimated to stop, it can be said that crack closure is caused by microbial healing.

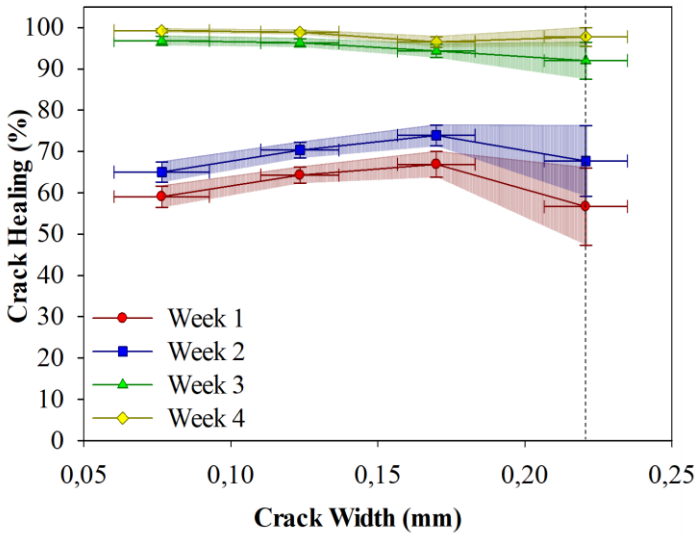

Figure 4. 37. Weekly change of microbial healing performance and determined self-healing capacity for Bio-1.25% samples. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

The self-healing limit of the cement composite, which heals itself with microbial means, was determined as 220 ± 15 µm after four weeks (Figure 4.37). Since a statistically significant healing of 90% and above in Bio-1.25% from the third week is obtained, the crack widths showing the best healing within different curing durations for Bio-1.25% and the self-healing capacity determined from the third week and it is presented in Table 4.11.

Table 4. 11. Self-healing capacity and healing percentages of 1 Bio-1.25% in different durations.

Duration	**Best healed crack widths (µm)**	**Crack healing ratio[1] (%)**
Week 1	<235	62±6
Week 2	<235	69±5
Week 3	<185	95±3
Week 4	<235	98±1

[1] As the crack healing ratio, the average of the healing percentages obtained in the given crack width ranges is presented.

4.5.8. Crack Healing Performance in Bio-1.50% Samples

Weekly crack healing graphs of samples containing bacteria in the form of biogranules at a rate of 1.50% by weight of cement are shown in Figure 4.38.

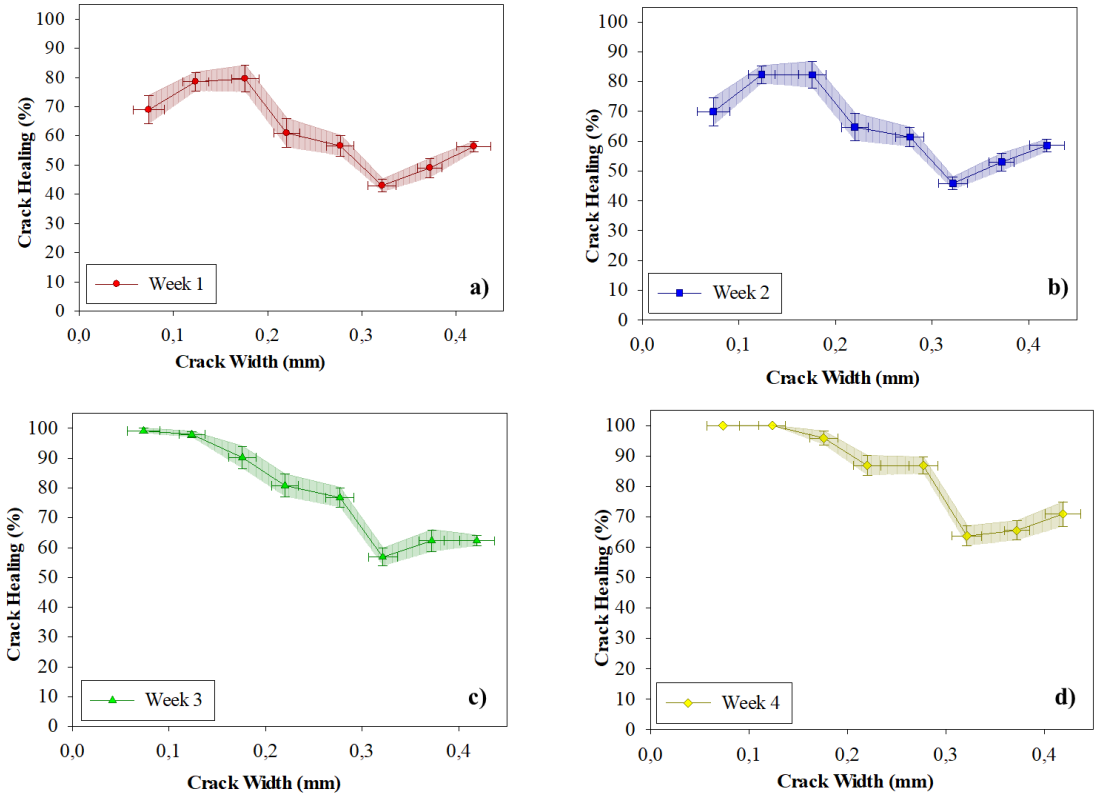

Figure 4. 38. Under water crack healing performances of Bio-1.50% samples (a) after 7 days; (b) at the end of the 14th day; (c) after 21 days; (d) after 28 days. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

The crack widths obtained in the bio-1.50% samples varied between 60 µm and 440 µm (Figure 4.38). In the Bio-1.50% samples, the highest crack healing performance was observed at the crack widths smaller than 190 µm at the end of the first week and it was determined that these crack widths healed by 70% to 80% (Figure 4.38-a). In the second week, the healing ratio remained constant in crack widths smaller than 90 µm, and an increase of approximately 5% was observed in the closure rate of all other crack widths (Figure 4.38-b). However, it was determined that there was no statistically significant change between the percentages of healing in the second week and the percentages in the first week ($p = 0.05$). In the third week, crack closure percentages showed a statistically significant increase in all observed crack widths ($p = 0.05$). Due to this increase, 90% and

more crack closure was obtained in cracks up to 190 µm (Figure 4.38-c). Closure in the cracks continued in the fourth week and 90% and more healing was obtained in the cracks up to 290 µm (Figure 4.38-d). It has been determined that the percentage of healing in wider cracks is around 70%. A representative visual of the widest range of microbial healing cracks in the Bio-1.50% sample is given in Figure 4.39.

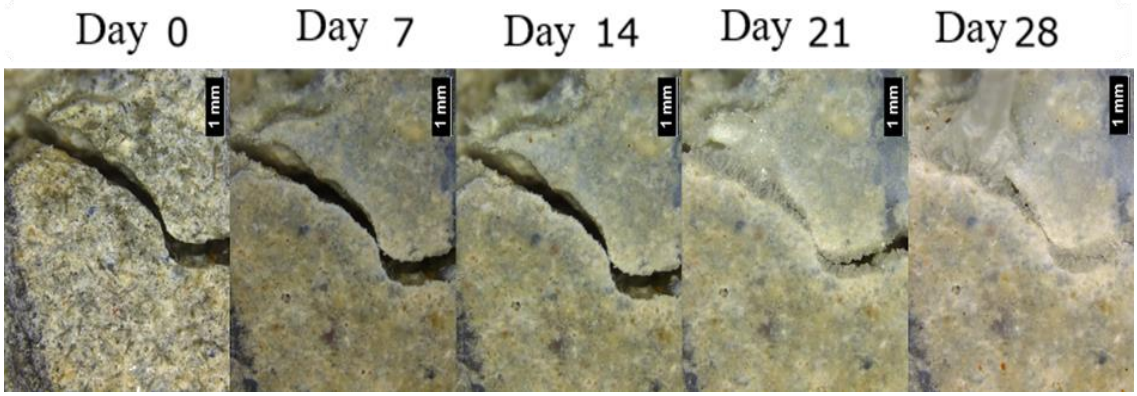

Figure 4. 39. Micrographs showing microbial healing in Bio-1.50% sample.

In Figure 4.40, 4-week crack healing performance and self-healing capacity obtained in Bio-1.50% samples are given. Bio-1.50% has been included in the self-healing cement composite class within the scope of the thesis, as over 90% improvement is achieved in crack widths up to 290 µm at the end of the curing period.

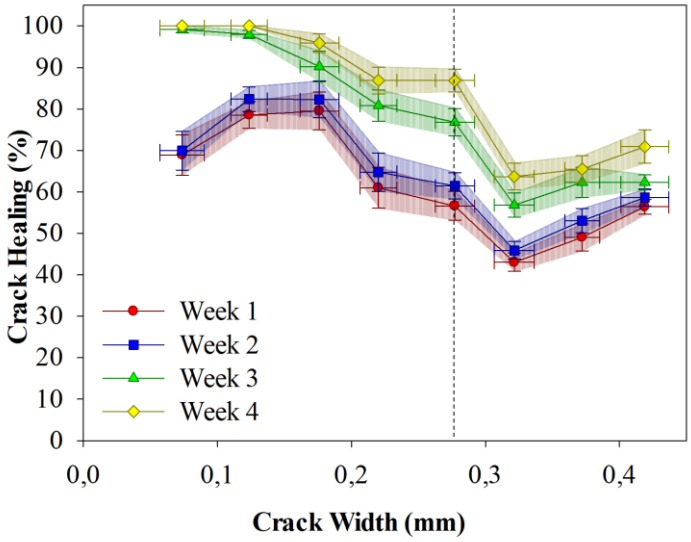

Figure 4. 40. Weekly change of microbial healing performance and determined self-healing capacity for Bio-1.50% samples. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

As in the other tested samples, in Bio-1.50%, the performances of the first two weeks and the performances afterwards are separated from each other. After the second week in which autogenous healing is estimated to stop, it can be said that crack closure is caused by microbial healing. The self-healing limit of the cement composite, which heals itself with microbial means, was determined as 275 ± 15 μm after four weeks (Figure 4.40). In the Bio-1.50% sample, as in the Bio-1.25 sample, a statistically significant healing of 90% and above was obtained from the third week, showing the best healing within different curing durations for the Bio-1.50% sample. Crack widths and self-healing capacities determined from the third week are presented in Table 4.12.

Table 4. 12.Self-healing capacity and percentages of Bio-1.50% samples at different durations.

Duration	**Best healed crack widths (μm)**	**Crack healing ratio[1] (%)**
Week 1	<190	76±7
Week 2	<190	78±7
Week 3	<190	96±5
Week 4	<290	93±6

[1] As the crack healing ratio, the average of the healing percentages obtained in the given crack width ranges is presented.

4.5.9. Crack Healing Performance in Bio-1.75% Samples

Weekly crack healing graphs of samples containing bacteria in the form of biogranules at 1.75% by weight of cement are shown in Figure 4.41.

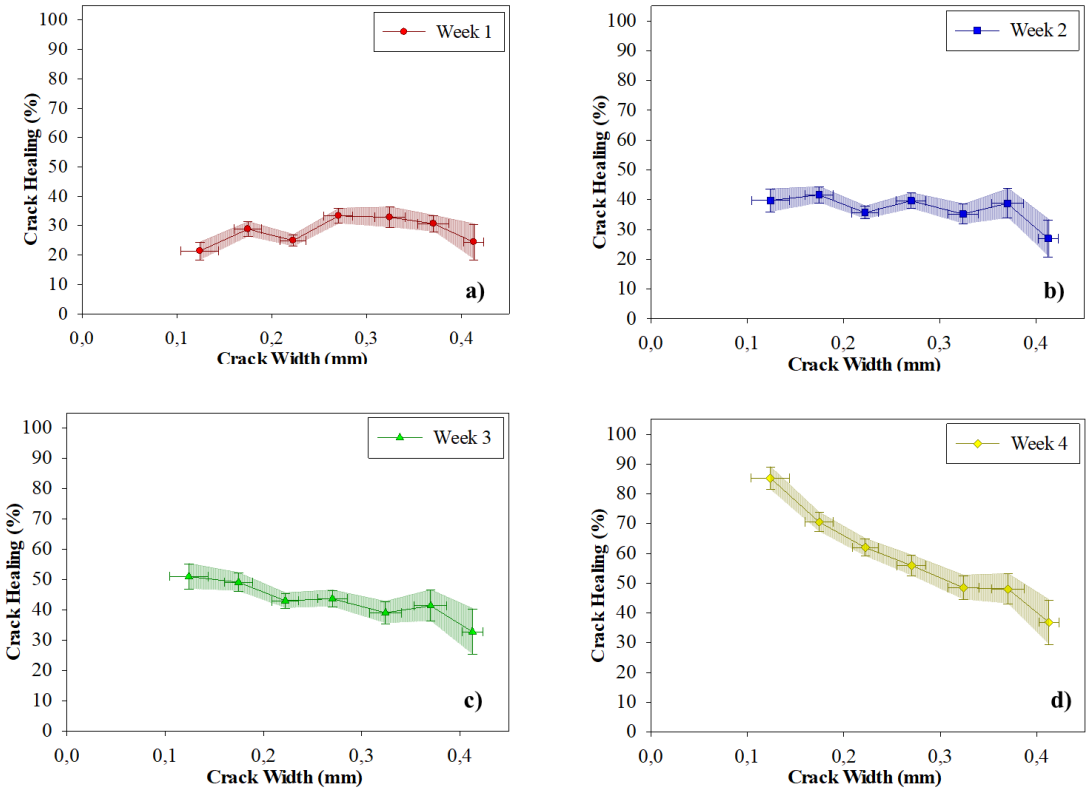

Figure 4. 41. Under water crack healing performances of Bio-1.75% samples (a) after 7 days; (b) at the end of the 14th day; (c) after 21 days; (d) after 28 days. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

The crack widths obtained in the Bio-1.75% samples varied between 100 µm and 420 µm. In the Bio-1.75% samples, healing performances varying between 25 and 35% were obtained in the first week, being similar in all crack widths ($p = 0.05$) (Figure 4.41-a). At the end of the second week, healing ratios increased in all crack widths and it was determined that the two-week crack healing performances varied between 30% and 40% (Figure 4.41-b). By the end of the third week, the healing percentages, which increased slightly more, did not show a statistically significant difference in all observed crack widths and varied between 40% and 50% (Figure 4.41-c). It was observed that the best healing crack widths at the end of four weeks were between 85 ± 4% and less than 150 µm (Figure 4.41-d). Since the healing efficiency obtained is very close to 90%, it has been decided to examine cracks smaller than 150 µm in smaller intervals. When the smaller crack widths were examined, it was observed that the cracks smaller than 120 µm healed by 92 ± 5%, and the cracks between 120-150 µm improved by 81 ± 5%. However,

since 23 data in the section up to 120 µm and 42 data between 120-150 µm are examined in this analysis, the data are not presented separately in Figure 4.41, taking into account the criterion of at least 60 data points specified in the thesis. There are microbial self-healing concrete studies in the literature where the self-healing capacity of mortars is determined using less data (n≥5) for crack gaps of 50 µm [25], [26], [29], [95]. Based on these studies, the self-healing capacity for Bio-1.75% has been determined as 100 ± 15 µm, with over 90% healing efficiency, and the healing duration as 4 weeks. From the specified limit value, it has been observed that as the crack width increases, the healing percentage of the cracks decreases linearly up to 50%. A representative visual of the crack width of less than 120 µm where microbial improvement is obtained in the Bio-1.75% samples and the crack gaps of approximately 200 µm where more than 90% improvement cannot be achieved is given in Figure 4.42.

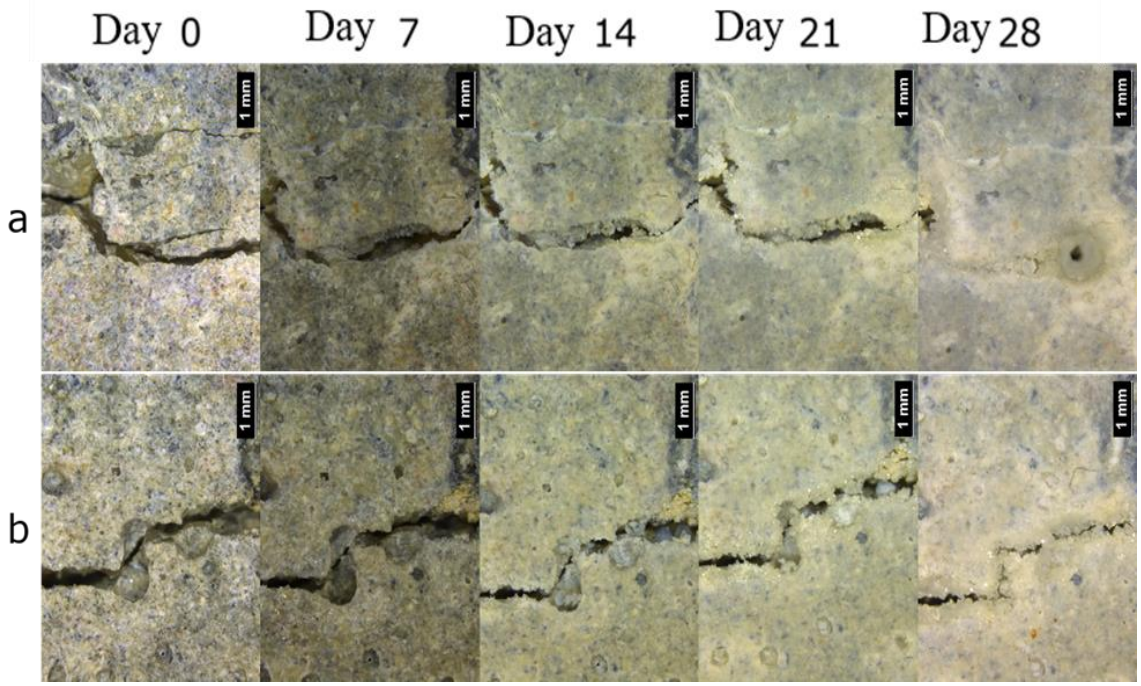

Figure 4. 42. In the Bio-1.75%; a) narrow crack micrographs where microbial healing is achieved; b) micrographs of cracks larger than 150 µm with healing below 90%.

In Figure 4.43, 4-week crack healing performance and self-healing capacity obtained in Bio-1.75% samples are given. Bio-1.75% was included in the self-healing cement composite class within the scope of this thesis, as over 90% healing was achieved in crack widths up to 120 µm at the end of the curing period.

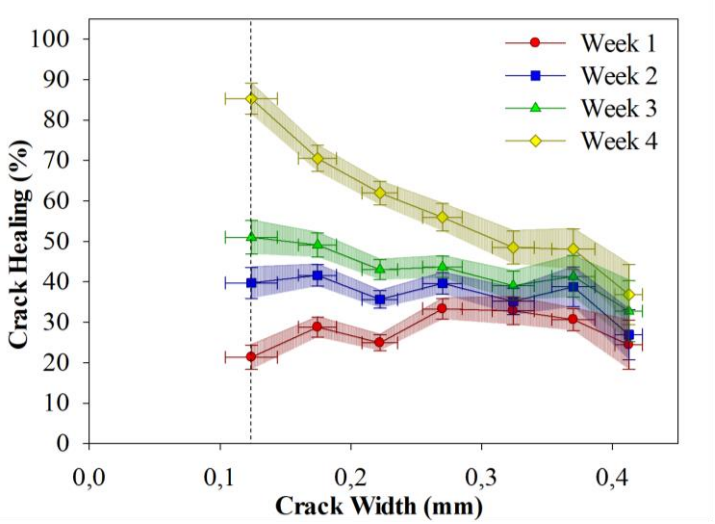

Figure 4. 43. Weekly change of microbial healing performance and determined self-healing capacity for Bio-1.75% samples. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

As with other tested bio-mortar samples, autogenous healing and microbial healing can be clearly distinguished in the Bio-1.75% sample. After the second week in which autogenous healing is estimated to stop, it can be said that crack closure is caused by microbial healing. Since there was no crack healing of 90% or more in the first three weeks in the Bio-1.75% sample, the crack widths that healed the most in different curing times and the highest crack healing percentages seen in these cracks were determined and presented in Table 4.13.

Table 4. 13.Crack widths, self-healing capacity and healing percentages of the Bio-1.75% sample that showed the highest healing at different durations.

Duration	**Best healed crack widths (µm)**	**Crack healing ratio[1] (%)**
Week 1	<420	28±5
Week 2	<420	37±6
Week 3	<420	43±7
Week 4	<120	92±5

[1] As the crack healing ratio, the average of the healing percentages obtained in the given crack width ranges is presented.

4.5.10. Crack Healing Performance in Bio-2.00% Samples

Weekly crack healing graphs of samples containing bacteria in the form of biogranules at a rate of 2.00% by weight of cement are shown in Figure 4.44.

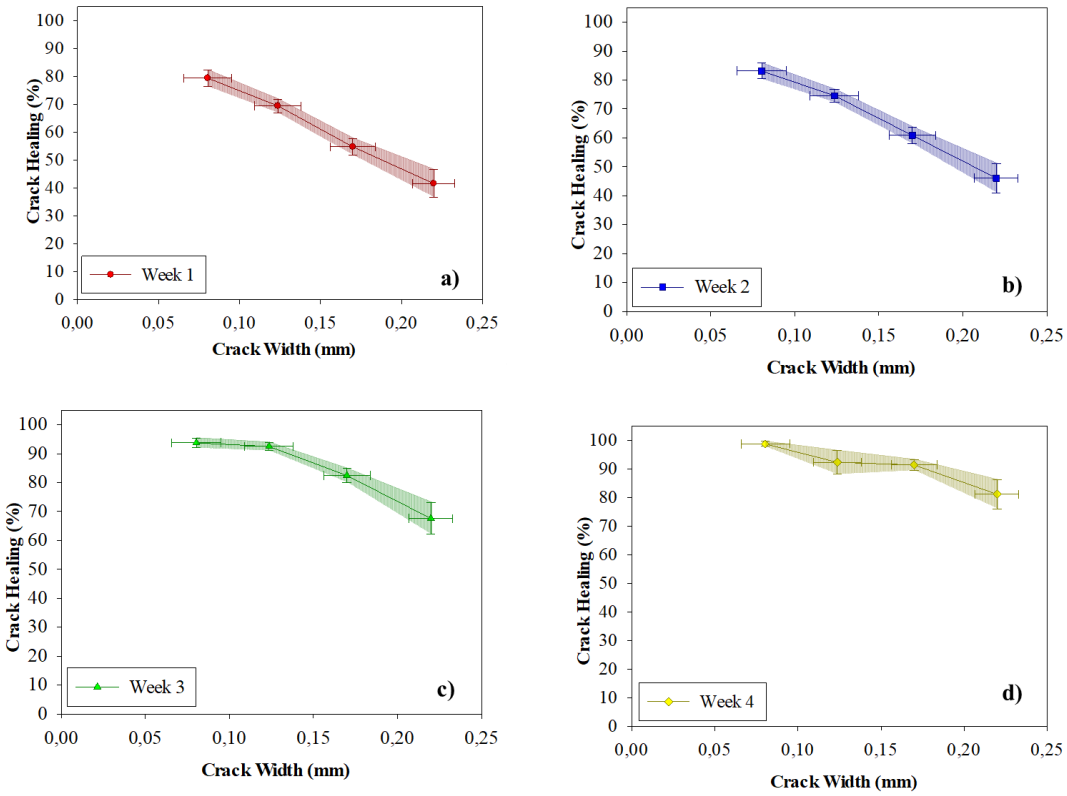

Figure 4. 44. Under water crack healing performances of Bio-2.00% samples (a) after 7 days; (b) at the end of the 14th day; (c) after 21 days; (d) after 28 days. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

The crack widths obtained in the Bio-2.00% samples varied between 65 µm and 235 µm. At the end of the first week at Bio- 2.00%, the highest crack healing performance was observed at crack widths less than 140 µm, and it was determined that these crack widths healed at rates varying between 70% and 80% (Figure 4.44-a). At the end of the second week, an increase of approximately 5% was observed in the healing percentages of all observed crack widths (Figure 4.44-b). However, there was no statistically significant difference between the healings obtained at the end of the first week and the second week ($p = 0.05$). Crack closure percentages showed a more serious increase in the third week and healing over 90% was obtained in crack widths smaller than 140 µm (Figure 4.44-c). All crack widths increased in the fourth week and the maximum crack width healed after

four weeks was recorded as 185 µm. It has been determined that the healing percentage in wider cracks is below 90%. A representative visual of the widest crack range that heals microbially in the Bio-2.00% sample is given in Figure 4.45.

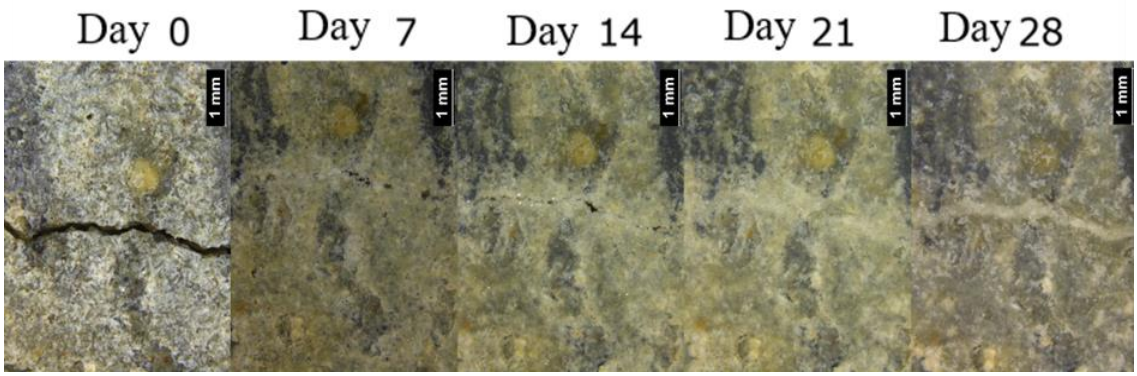

Figure 4. 45. Micrographs showing microbial healing in Bio-2.00% sample.

Figure 4.46 shows the 4-week crack healing performance and self-healing capacity obtained in Bio-2.00% samples. Bio-2.00% sample has been included in the self-healing cement composite class within the scope of this thesis, as over 90% healing is recorded in crack widths up to 185 µm at the end of the curing period.

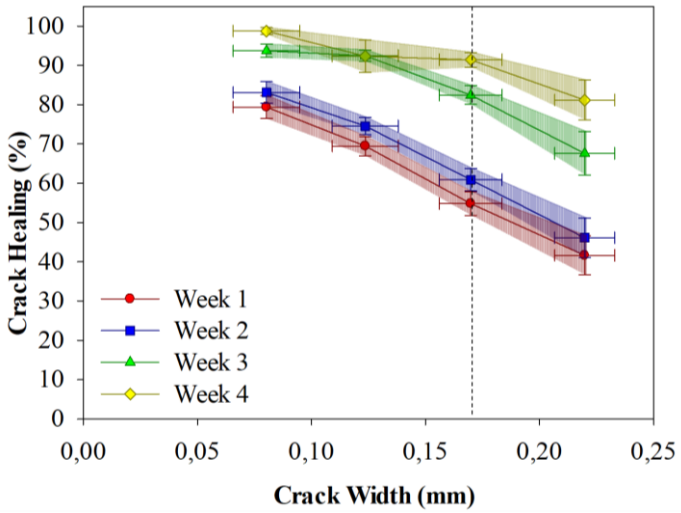

Figure 4. 46. Weekly change of microbial healing performance and determined self-healing capacity for Bio-2.00% samples. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

As with other tested bio-mortar samples, autogenous healing and microbial healing can be clearly distinguished in Bio-2.00% samples. It can be said that autogenous healing occurs predominantly in the first two weeks, and microbial healing becomes prominent

after the third week (Figure 4.46). The self-healing limit of the cement composite, which heals itself with microbial means, was determined as 170 ± 15 µm after four weeks (Figure 4.46). In Bio-2.00%, as in the Bio-1.50% and Bio-1.25 samples, a statistically significant healing of 90% and above was obtained from the third week. On the other hand, the crack widths showing the best healing in different curing durations for the Bio-2.00% sample and the self-healing capacities determined from the third week are presented in Table 4.14.

Table 4. 14.Crack widths, self-healing capacity and healing percentages of the Bio-2.00% sample that showed the highest healing at different durations.

Duration	**Best healed crack widths (µm)**	**Crack healing ratio[1] (%)**
Week 1	<140	74±6
Week 2	<140	79±5
Week 3	<140	93±2
Week 4	<185	94±4

[1] As the crack healing ratio, the average of the healing percentages obtained in the given crack width ranges is presented.

4.5.11. Crack Healing Performance in Bio-2.25% Samples

Weekly crack healing graphs of samples containing bacteria in the form of biogranules at a rate of 2.25% by weight of cement are shown in Figure 4.47.

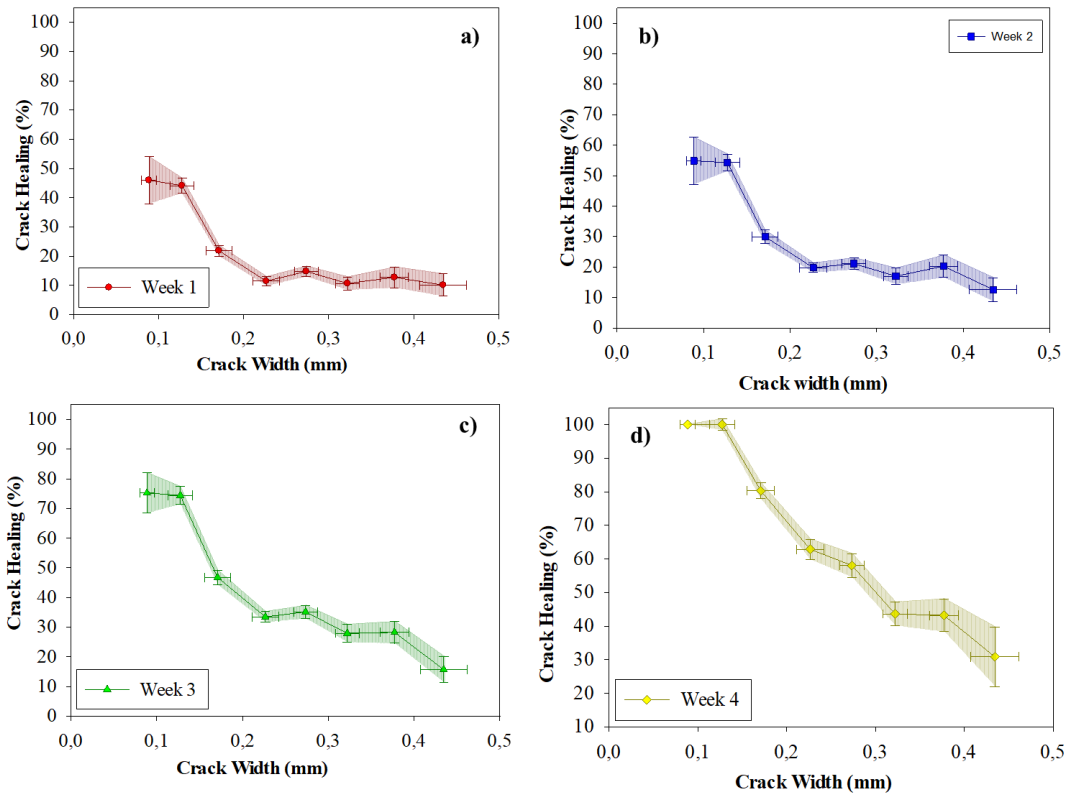

Figure 4. 47.Under water crack healing performances of Bio-2.25% samples (a) after 7 days; (b) at the end of the 14th day; (c) after 21 days; (d) after 28 days. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

The crack widths obtained in the Bio-2.25% samples varied between 80 µm and 460 µm (Figure 4.47). In the Bio-2.25% samples, the highest crack healing performance was observed at the crack widths less than 140 µm at the end of the first week and it was determined that these crack widths healed by approximately 45% (Figure 4.47-a). At the end of the second week, an increase of approximately 10% was observed in the healing percentages of all observed crack widths (Figure 4.47-b). This observed increase was found to be statistically significant in all observed crack widths except for crack widths less than 100 µm and crack widths varying between 400–460 µm ($p = 0.05$). Crack closure percentages showed a serious increase, approximately 20% in cracks smaller than 185 µm in the third week, and an improvement of 75% was obtained in crack widths less than 140 µm (Figure 4.47-c). Of the other crack intervals, 50% healing was obtained in the crack range of 155–185 µm and 30% in the crack range of 200–400 µm (Figure 4.47-c). In the fourth week, a 30% increase was obtained in crack spacing up to 290 µm and a

crack healing percentage over 90% was obtained in crack widths less than 140 µm (Figure 4.47-d). It has been determined that the healing percentage in wider cracks is below 90% and it decreases as the crack width increases. A representative visual of the widest range of microbial healing cracks in the bio-2.25% samples is given in Figure 4.48.

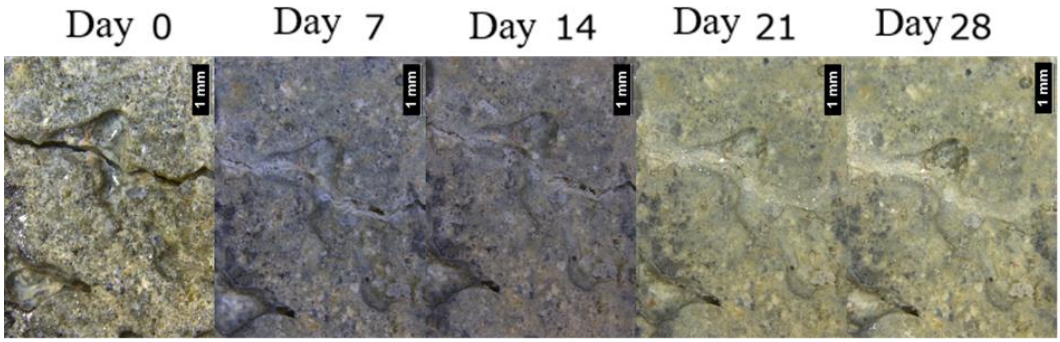

Figure 4. 48. Micrographs showing microbial healing in Bio-2.25% sample.

In Figure 4.49, 4-week crack healing performance and self-healing capacity obtained in Bio-2.25% samples are given. At the end of the curing period, as over 90% healing is recorded in crack widths up to 140 µm, Bio-2.25% sample is included in the self-healing cement composite class within the scope of this thesis. As with other bio-mortar samples tested, the increase in crack closure percentages between weeks in the Bio-2.25% sample was higher in the third and fourth week (Figure 4.49). Therefore, autogenous healing and microbial healing can be clearly distinguished in Bio-2.25% samples.

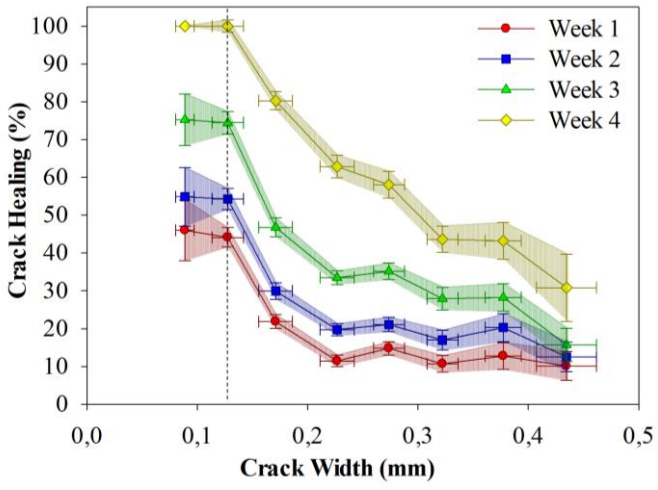

Figure 4. 49.Weekly change of microbial healing performance and determined self-healing capacity for Bio-2.25% samples. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

The self-healing limit of the cement composite, which heals itself with microbial means, was determined as 130 ± 10 μm after four weeks (Figure 4.49). In the Bio-2.25% sample, as in the Bio-0.50% and Bio-1.75% samples, there was no crack healing of 90% or more in the first three weeks, thus the crack widths that healed the most within different curing durations and the highest crack healing percentages seen in these cracks were determined and presented in Table 4.15.

Table 4. 15. Crack widths, self-healing capacity and healing percentages of the Bio-2.25% sample that showed the highest healing at different durations.

Duration	**Best healed crack widths (μm)**	**Crack healing ratio[1] (%)**
Week 1	<140	45±6
Week 2	<140	55±5
Week 3	<140	75±5
Week 4	<140	99±1

[1] As the crack healing ratio, the average of the healing percentages obtained in the given crack width ranges is presented.

4.5.12. Crack Healing Performance in Bio-2.50% Samples

Weekly crack healing graphs of samples containing 2.50% biogranule form bacteria by weight of cement, which is the maximum bacterial dose tested in self-healing performance tests, are shown in Figure 4.50.

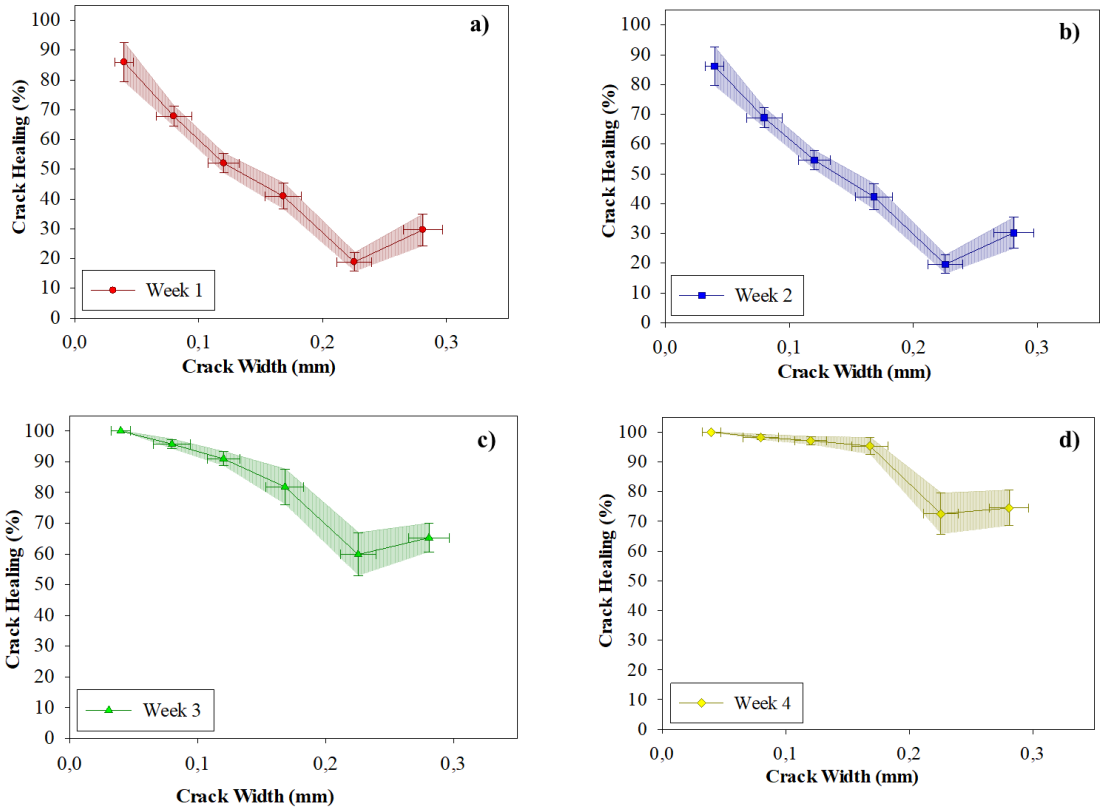

Figure 4. 50. Under water crack healing performances of Bio-2.50% samples (a) after 7 days; (b) at the end of the 14th day; (c) after 21 days; (d) after 28 days. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

The crack widths obtained in the Bio-2.50% samples varied between 30 µm and 295 µm (Figure 4.50). In the Bio-2.50% samples, the highest crack healing performance was observed at the crack widths less than 50 µm at the end of the first week and it was determined that these crack widths healed by approximately 85% (Figure 4.50-a). At the end of the second week, there was no statistically significant change in the healing percentages of all observed crack widths ($p = 0.05$). Crack closure percentages showed a significant increase in the third week and healing over 90% was obtained in crack widths less than 135 µm (Figure 4.50-c). All crack widths increased in the fourth week and the maximum crack width that healed after four weeks was recorded as 185 µm (Figure 4.50-d). It has been determined that the percentage of healing in wider cracks has decreased up to 70%. A representative visual of the widest range of microbial healing cracks in the Bio-2.50% sample is given in Figure 4.51.

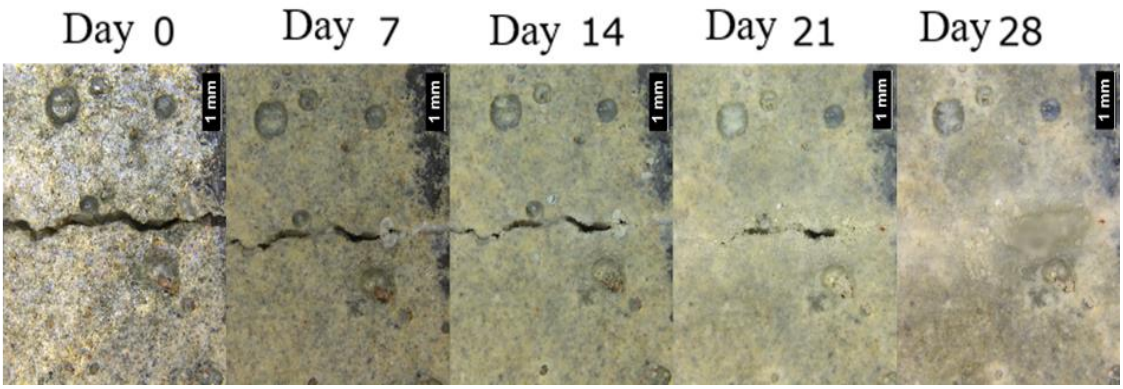

Figure 4. 51. Micrographs showing microbial healing in Bio-2.50% sample.

In Figure 4.52, 4-week crack healing performance and self-healing capacity obtained in Bio-2.50% samples are given. At the end of the curing period, over 90% healing is achieved in crack widths up to 185 μm, the Bio-2.50% sample has been included in the self-healing cement composite class within the scope of this thesis.

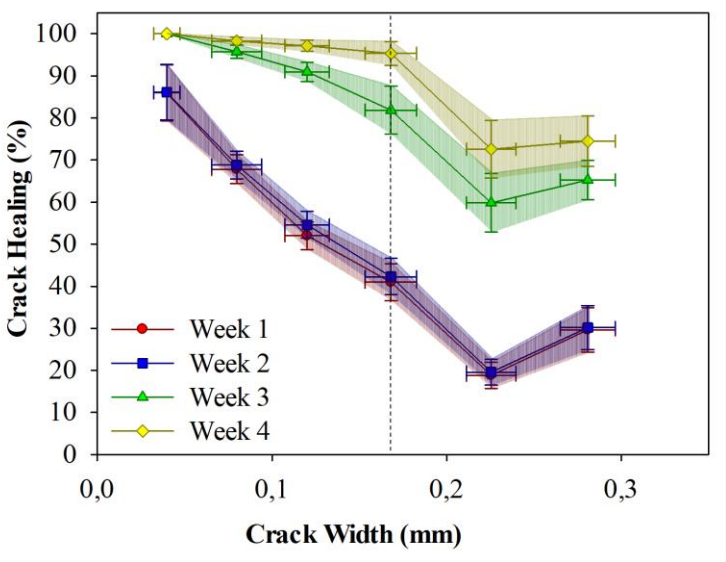

Figure 4. 52. Weekly change of microbial healing performance and determined self-healing capacity for Bio-2.50% samples. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

As with other tested bio-mortar samples, autogenous healing and microbial healing can be clearly distinguished in the Bio-2.50% sample. It can be said that autogenous healing occurs in the first two weeks, and microbial healing begins after the third week. The self-healing limit of the cement composite, which heals itself with microbial means, was determined as 170 ± 15 μm after four weeks (Figure 4.52). In the Bio-2.50% sample, as

in the Bio-2.00%, Bio-1.50% and Bio-1.25 samples, a statistically significant healing of 90% and above was obtained from the third week. On the other hand, the crack widths showing the best healing in different curing durations for the Bio-2.50% sample and the self-healing capacities determined from the third week are presented in Table 4.16.

Table 4. 16. Crack widths, self-healing capacity and healing percentages of the Bio-2.50% sample that showed the highest healing at different durations.

Duration	**Best healed crack widths (µm)**	**Crack healing ratio[1] (%)**
Week 1	<50	86±7
Week 2	<50	86±7
Week 3	<135	94±4
Week 4	<185	97±2

[1] As the crack healing ratio, the average of the healing percentages obtained in the given crack width ranges is presented.

4.5.13. Effect of Increasing Bacterial Dose on Self-healing Capacity by Microbial Means

Within the scope of the thesis study, 10 different bio-mortar mixtures (containing 0.25% - 2.50% bacteria by weight of cement) were examined in order to investigate the effect of bacterial dosage in biogranule form.

All of the bio-mortar mixes examined were included in the class of cementitious composites that heals themselves by microbial means, as 90% or more healing was achieved within the curing time (maximum 4 weeks). The self-healing performances of all mixtures were compared among themselves in order to determine the bacterial dose in the form of biogranules that give the most efficient results. Self-healing capacities and durations to reach self-healing capacity were compared. (Table 4.17). While determining the self-healing capacity, crack widths that healed at least 90% during the curing period were taken into account, and lower healing percentages were not taken into account. Since the healing behaviour in all samples in the first week was inconsistent and unpredictable

due to autogenous healing, the evaluations were made after the second week performances.

Table 4. 17. The self-healing capacities achieved in different bio-mortar samples and the durations to reach the self-healing capacity.

Mixture	Week 2		Week 3		Week 4	
	Self-healing capacity[1] (µm)	Healing ratio (%)	Self-healing capacity (µm)	Healing ratio (%)	Self-healing capacity (µm)	Healing ratio (%)
Bio-0.25%	550±30	95±5	800±30	97±3	800±30	98±2
Bio-0.50%	-	-	-	-	520±50	91±5
Bio-0.75%	125±15	99±2	310-525	93±5	500±25	95±5
Bio-1.00%	175±15	93±5	265±15	94±5	320±10	96±5
Bio-1.25%	-	-	170±15	95±3	220±15	98±1
Bio-1.50%	-	-	175±15	96±5	275±15	93±6
Bio-1.75%	-	-	-	-	100±15	92±5
Bio-2.00%	-	-	125±15	93±2	170±15	94±4
Bio-2.25%	-	-	-	-	130±10	99±1
Bio-2.50%	-	-	120±15	94±4	170±15	97±2

[1]Crack widths, determined as self-healing capacity, improved by at least 90% during the curing time, lower healing percentages were not taken into account.

Biogranule content in microbial self-healing cement composites prepared within the scope of this thesis consists of nitrate reducing spores as described in Section 4.1.2 and shown in Figure 4.11-b. Water, pH drop and the amount of water-soluble nutrients play a role in the revival of bacterial spores. While spores of bacteria come alive and produce new cells, carbon and nitrogen sources (calcium formate and calcium nitrate) are transformed into molecules that form the cell structure by anabolic reactions. In the biomineralization process, the catabolic reactions that the bacteria convert to carbon dioxide and carbonate by oxidizing the carbon source with nitrate reduction for energy production play a role. Microbially induced calcium carbonate precipitation is also a biomineralization process and one of the four main factors affecting the amount of

calcium carbonate precipitated is the concentration of dissolved inorganic carbon (dissolved carbon dioxide and carbonate) [49]. Therefore, as the amount of spores in the mortar mixture increases, there is an increase in the amount of nutrients used for regeneration after crack formation. The increase in the amount of nutrients used in anabolic reactions means that the nutrient in the environment is mostly used in the cell structure instead of calcium carbonate precipitation. Since the basis of crack healing by microbial means is the calcium carbonate precipitation triggered by bacteria, the decrease in the amount of precipitated calcium carbonate will negatively affect the crack repair performance. In this thesis study, it is thought that as the dosage of bacteria in the mortar increases, the main reasons for achieving lower self-healing capacities in the same curing durations are the use of nutrients to germinate spores and it is thought that the amount of nutrients expected to be used for calcium carbonate precipitation decreases with the increase in the initial spore amount.

The results revealed that the increase in the bacterial dose in the form of biogranules in the mortar mixture negatively affected the healing rate and healing capacity (Table 4.17). The highest healing capacity was obtained in the Bio-0.25% sample with 800 ± 30 μm. In addition, the mixture that reached the healing capacity the fastest was Bio-0.25% within 3 weeks (Table 4.17). The performance of the Bio-0.25% sample is promising to maintain the durability of the cement-binder system with rebars in terms of avoiding corrosion by preventing the ingress of external elements thanks to microbial calcium carbonate precipitation in cracks. It was determined that the four-week healing capacities of the higher doses of Bio-0.50% and Bio-0.75% samples were not statistically different and were around 500 μm ($p = 0.05$) (Table 4.17). In the Bio-1.00% sample, the four-week healing capacity decreased slightly, reaching around 300 μm (Table 4.17). It was observed that the four-week healing capacity of the Bio- 1.25% and Bio- 1.50% samples also fell below 300 μm (Table 4.17). It has been observed that four-week healing capacity falls below 200 μm when more than 1.50% of the weight of cement is used in the form of biogranules (Table 4.17). Similarly, it was observed that the healing capacities determined for the three-week curing period decreased from Bio-0.25% to Bio-2.50% (Table 4.17). When the healing ratios were examined, it was determined that samples with an initial bacterial dose of up to 1.00% healed faster than samples containing higher doses of bacteria. It might be related with germination process of bacteria. In a study, it is stated that the dormant bacterial spores have been used to increase survival within the

cementitious matrix for the self-healing systems. Nevertheless, a slow $CaCO_3$ precipitation has been demonstrated by *B. sphaericus* spores as vegetative cells must germate before precipitation could begin [136]. Based on this and obtained results in this thesis study, it might be stated that more than 1.00% biogranules contained specimens might use a part of available nutrients for germination. In this study, to investigate the effects of biogranules dosages in cement-binder systems directly, the amounts of nutrients in samples were kept stable for each specimen. Since higher amounts of biogranules need a higher amount of nutrients to germinate bacteria, utilized nutrients in the study could be inadequate to initiate effective calcium carbonate precipitation. Because after germination, bacteria could have been consumed a lot of nutrients in high doses of bacteria incorporated specimens, induced calcium carbonate precipitation throughout microbial activity might be lower than specimens with fewer doses of bacteria introduced. Likewise, another study revealed that hydrolysis of the bacterial urea and organic carbon aerobic oxidation demand oxygen to trigger bacterial activity (spore germination). This could be a restrictive factor for deep crack healings, or as a final electron accepter to activate and maintain microbial activity [40]. Although denitrifying agents which can utilize nitrate (NO_3^-) as an alternative acceptor electron for organic carbon oxidation under oxygen-limited conditions [88], were used in this thesis study, obtained crack widths in specimens that prepared with higher dosages of biogranules were smaller compared to specimens produced with biogranules at the rate of lower than 1.00%. This might be related to obtaining less water ingress or a less soluble environment for activating bacteria. However, these can not be certain results because Bio-0.50% and Bio-0.75% samples had also crack widths up to 500 μm.

It is thought that the difference in activity between spores and vegetative bacteria at different pH values increases the rate of use of nutrients by the spores. Within the scope of this thesis, the pH values of the nutrient solution remained around 10.0 during the production of biogranules, while the pH values in the reactor were between 9.5 - 10 in the anoxic period and 9.0 - 9.5 in the aerobic period (Figure 4.1-a). There is no need to create optimum conditions for spore cells to regenerate, spores begin to revive when there are pH and nutrient conditions available, where bacteria can carry out their metabolic activities after healing [136]. Therefore, according to the production pH values, it is possible for spores to use nutrients in anabolic reactions at pH values of 10,0–10,5 and the revitalized bacteria to oxidize carbon with low efficiency. On the other hand, effective

carbon oxidation is expected to occur under conditions where bacteria are accustomed to reactor operation and pH values fall below 10 (Figure 4.1-a, c). Weekly pH measurements were made while the cracked samples were cured. According to the pH measurements made, it was determined that the pH values of all samples varied between 10.0 and 10.5 at the end of the first week. Therefore, it can be said that sports started to revive at the end of the first week. At the end of the second week, it was determined that the pH values changed between 9.5 and 10.5. Thus, the effect of microbial healing was more clearly seen in all samples except Bio-0.25% from the 3rd week. The reason for this is that with the decreasing pH, nutrients are used for energy production more effectively and calcium carbonate precipitation accelerates due to the catabolic activity of bacteria. Similar behaviour of spores has been reported in previous studies [136]. In samples containing high doses of bacterial spore, the nutrient leaking into the environment is consumed mostly during the revitalization of the spores and therefore less food is left to be used in catabolic activities in the remaining process, when the amount of initial spores used in the mortar is increased, a decrease in the self-healing capacity of the cement composite has been observed.

4.6. The Minimum Amount of Bacteria Required to Obtain a Cementitious Composite that Heals Itself by Microbial Means

Within the scope of the thesis, samples were also prepared to determine the minimum amount of bacteria in the form of biogranules required to obtain a cementitious composite that heals itself by microbial means. The success criterion was defined as obtaining a higher healing capacity than the healing capacity obtained in the reference and control samples or reaching the same capacity in a shorter time than autogenous healing. In the bacterial doses in the form of biogranules tested at intervals of 0.25% by weight of cement, the lowest dose where the microbial healing was achieved effectively was determined as Bio-0.25%. Therefore, in order to determine whether an healing was achieved at doses lower than this dose, Bio-0.05% and Bio-0.10% samples were prepared like the previous samples and their healing performances were compared against the healing performances of the reference and control samples.

4.6.1. Crack Healing Performance in Bio-0.05% Samples

Weekly crack healing graphs of samples containing bacteria in the form of biogranules at the rate of 0.05% by weight of cement, which is the minimum bacteria dose tested within the scope of the thesis, are shown in Figure 4.53.

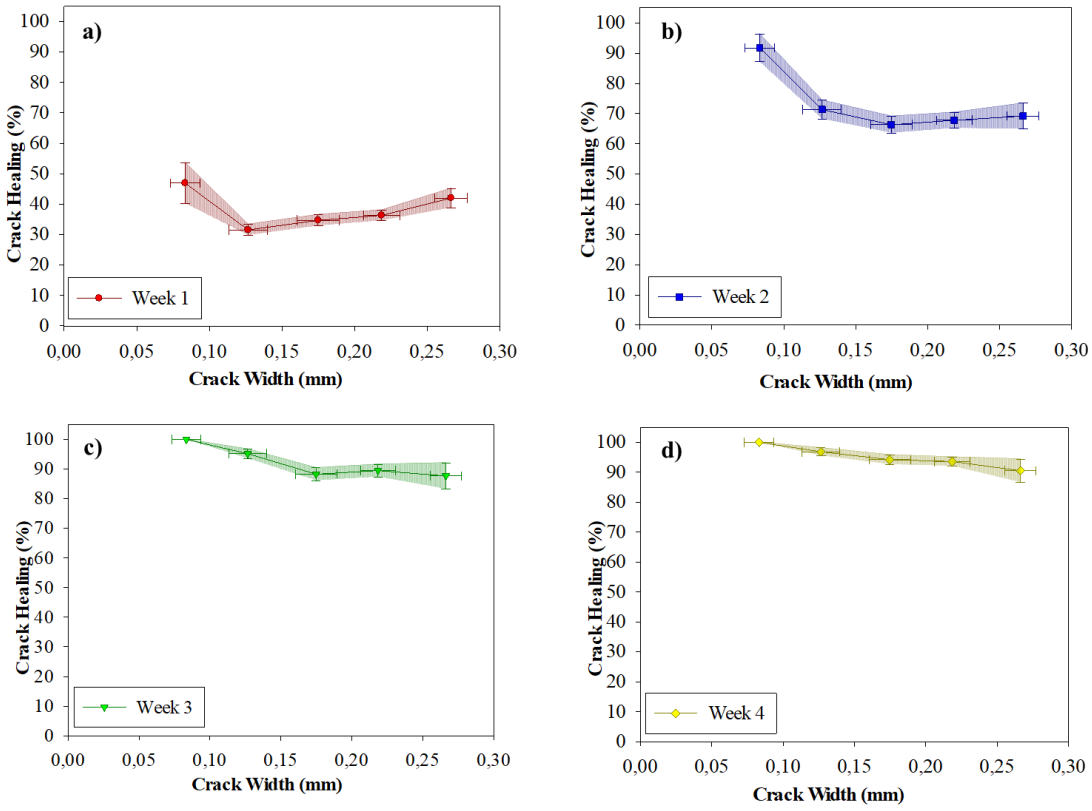

Figure 4. 53.Under water crack healing performances of Bio-0.05% samples (a) after 7 days; (b) at the end of the 14th day; (c) after 21 days; (d) after 28 days. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

The crack widths in the Bio-0.05% samples were obtained between 75 µm and 275 µm in a way to provide comparison with reference samples and control samples (Figure 4.53). In the Bio-0.05% samples, the highest crack healing performance was observed at the crack widths less than 95 µm at the end of the first week and it was determined that these crack widths healed by approximately 50% (Figure 4.53-a). Similar to the reference samples, crack widths between 255–275 µm showed a 40% healing in the first week (Table 4.5). At the end of the second week, an increase of approximately 50% was observed in the healing ratio of crack widths smaller than 140 µm, and closure percentages of over 90% were obtained in crack widths less than 95 µm (Figure 4.53-b).

Crack closure percentages continued to increase in the third week and healing over 90% was obtained in all observed crack widths (Figure 4.53-c). A slight increase was recorded in all crack widths observed in the fourth week and the maximum crack width that healed after four weeks was recorded as 275 µm (Figure 4.53-d). A representative visual of the widest range of microbial healing cracks in the Bio-0.05% sample is given in Figure 4.54.

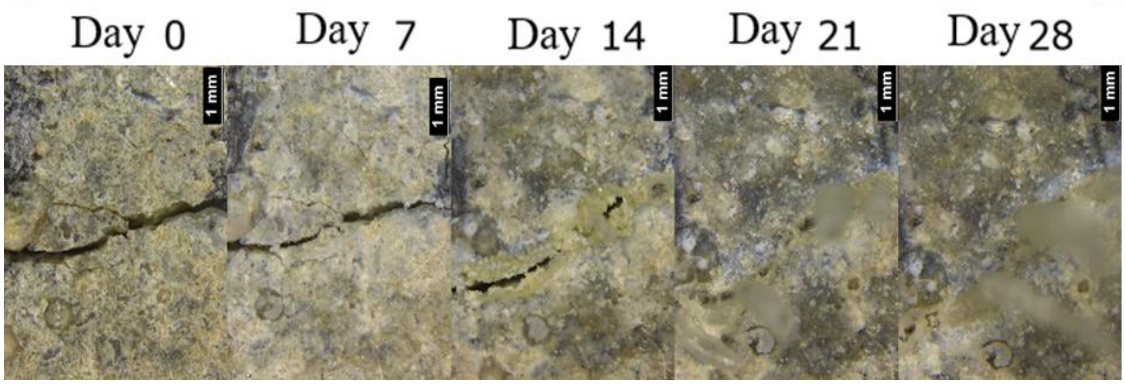

Figure 4. 54. Micrographs showing microbial healing in Bio-0.05% sample.

In Figure 4.55, 4-week crack healing performance and self-healing capacity obtained in Bio-0.05% samples are given. Since over 90% healing is achieved in crack widths up to 275 µm at the end of the curing period, Bio-0.05% has been included in the self-healing cement composite class within the scope of this thesis. The Bio-0.05% sample outperformed crack healing ratios and crack healing capacities obtained in both reference and control samples.

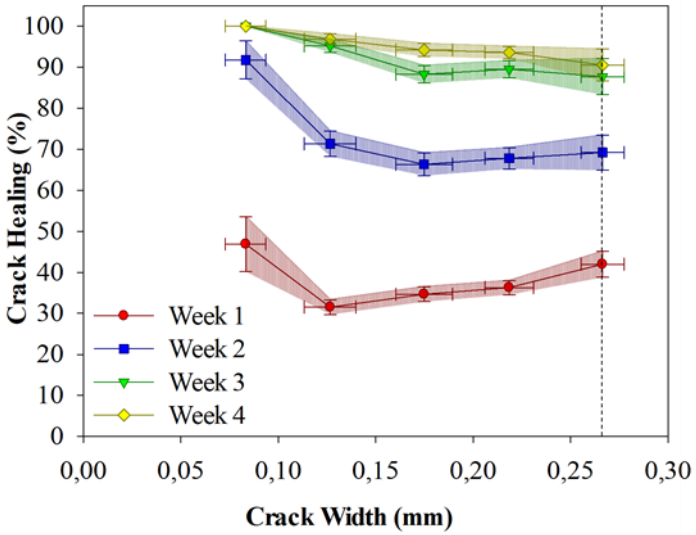

Figure 4. 55. Weekly change of microbial healing performance and determined self-healing capacity for Bio-0.05% samples. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

Bio-0.05% samples showed healing over 90% in all crack widths observed within 3 weeks, such as Bio-0.25 samples (Figure 4.55). Since the purpose of preparing Bio-0.05% is to compare it with reference and control samples, large crack widths have not been followed. Therefore, although the four-week healing limit for Bio-0.05% was determined as 275 μm within the scope of this study, it is recommended to examine higher crack intervals in future studies. Because the results obtained in this study, especially due to the similarity of the healing performance to the Bio-0.25% sample, indicate that the four-week healing limit may be above 275 μm. Crack widths showing the best healing in different curing durations for the Bio-0.05% sample and the self-healing capacities determined from the third week are presented in Table 4.18.

Table 4. 18. Crack widths, self-healing capacity and healing percentages of the Bio-0.05% sample that showed the highest healing at different durations.

Duration	**Best healed crack widths (μm)**	**Crack healing ratio[1] (%)**
Week 1	<95	47±7
Week 2	<95	92±5
Week 3	<275	91±5
Week 4	<275	94±3

[1] As the crack healing ratio, the average of the healing percentages obtained in the given crack width ranges is presented.

4.6.2. Crack Healing Performance in Bio-0.10% Samples

Within the scope of this study, Bio-0.05% samples and Bio-0.10% samples were also tested in order to determine the minimum amount of bacteria in the form of biogranules required to obtain a cementitious composite that heals itself by microbial means. Weekly crack healing graphs of samples containing bacteria in the form of biogranules at a rate of 0.10% by weight of cement are shown in Figure 4.56.

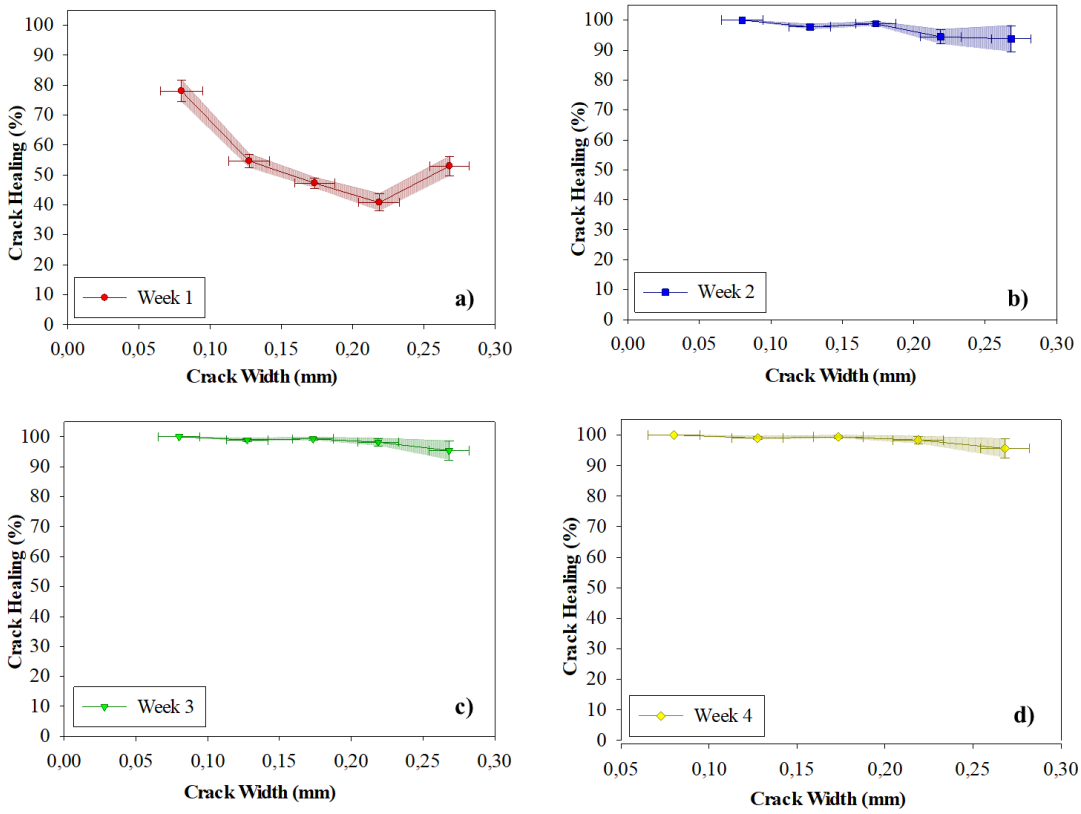

Figure 4. 56. Under water crack healing performances of Bio-0.10% samples (a) after 7 days; (b) at the end of the 14th day; (c) after 21 days; (d) after 28 days. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

The crack widths obtained in the Bio-0.10% samples were obtained between 65 and 280 µm in terms of comparison with reference and control samples (Figure 4.56). In the Bio-0.10% samples, the highest crack healing performance was observed at the crack widths less than 95 µm at the end of the first week and it was determined that these crack widths healed by approximately 80% (Figure 4.56-a). As in the Bio-0,25% samples, a healing over 90% was obtained in the crack widths less than 280 µm in the Bio-0,10% samples at the end of the second week (Figure 4.56-b). Since all observed cracks were closed at the end of three weeks, no statistically significant increase was observed in the crack closure percentages in the fourth week and the maximum crack width that healed after four weeks was recorded as 280 µm (Figure 4.56-c, d). A representative visual of the widest range of cracks that heals microbially in Bio-0.10% is given in Figure 4.57.

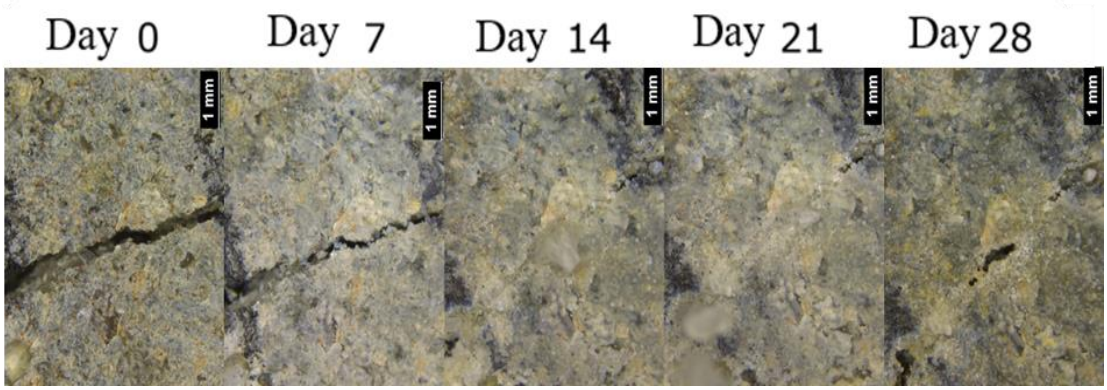

Figure 4. 57. Micrographs showing microbial healing in Bio-0.10% sample.

Figure 4.58 shows the 4-week crack healing performance and self-healing capacity obtained in Bio-0.10% samples. Bio-0.10% sample was included in the self-healing cement composite class within the scope of this study, as over 90% healing was recorded in crack widths up to 280 µm at the end of the curing period. The Bio-0.10% sample outperformed the crack healing ratios and crack healing capacities obtained in both the reference and control samples.

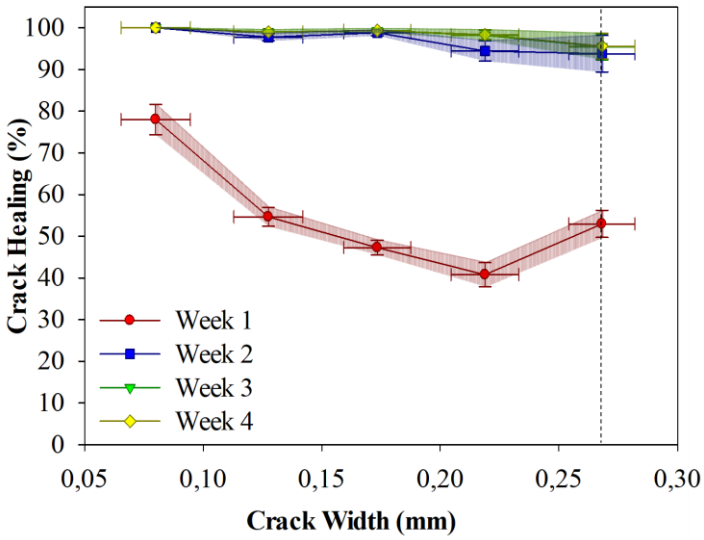

Figure 4. 58. Weekly change of microbial healing performance and determined self-healing capacity for Bio-0.10% samples. Transverse error bars indicate standard deviation, longitudinal error bars indicate standard error.

Crack widths showing the best healing in different curing durations for Bio-0.10% samples and self-healing capacities determined from the second week are presented in Table 4.19.

Table 4. 19. Crack widths, self-healing capacity and healing percentages of the Bio-0.10% sample that showed the highest healing at different durations.

Duration	Best healed crack widths (μm)	Crack healing ratio[1] (%)
Week 1	<95	78±4
Week 2	<280	97±3
Week 3	<280	98±2
Week 4	<280	98±2

[1] As the crack healing ratio, the average of the healing percentages obtained in the given crack width ranges is presented.

The healing performances of the Bio-0.10% samples were compared to both the reference and control samples and the healing performances of the Bio-0.05% and Bio-0.25% samples. It was determined that the healing performance of the Bio-0.10% samples was statistically significantly better than the autogenous healing in the reference and control samples at all curing times ($p = 0.05$). In addition, the healing performance obtained for the crack widths between 75-275 μm of Bio-0.10% samples in the first two weeks were statistically significantly better than the healing performance obtained in the same crack interval and the same time period Bio-0.05% samples ($p = 0.05$). It was determined that the healing performance of Bio-0.10% samples was more similar to Bio-0.25 sample compared to Bio-0.05%. Although it has been noted that for the same crack interval (65 - 280 μm), the healing performance of the Bio-0.25% sample in the first week was significantly better than the Bio-0.10% sample, for the same crack interval, there was no statistically significant difference in the crack healing performance of the mixture ($p = 0.05$). Therefore, it was not necessary to examine other doses between Bio-0.10% and Bio-0.25% in terms of healing performance.

Within the scope of this thesis study, although the four-week healing limit was determined as 280 μm for the Bio-0.10% sample as well as the Bio-0.05% sample, it would be useful to examine the larger crack intervals in future studies in terms of determining a more accurate self-healing capacity. Especially considering the similarity of the healing performance of Bio-0.10% to the Bio-0.25% samples more than Bio-0.05%, it can be said that the four-week healing limit is more than 280 μm. In the light of the results obtained,

it can be said that the minimum amount of bacteria in the form of biogranules required to obtain cement composite with microbial means is 0.05% by weight of cement. This dose is 10 times lower than the dose at which successful results were obtained by using biogranules in previous studies (bacteria in the form of biogranules at a rate of 0.50% by weight of cement). In addition, in this study, it was found that if the biogranule dose is decreased from 2.50% to 0.25% by weight of cement, there is an increase in the healing performances. The results obtained are promising in terms of reducing the cost of cementitious composites that heal themselves by microbial means containing biogranules. It was calculated the cost of self-healing cementitious composite with microbial means obtained by using bacteria in the form of biogranules and the cost of converting 1 m^3 normal concrete into self-healing bio-concrete as €194 [137]. The cost of self-healing concrete with microbial means calculated by taking the concrete unit cost as 85€/m^3 has been reported as ~ 280€/m^3 [137]. In this calculation, it was stated that 3.00% calcium nitrate and 2.00% calcium formate were used by weight of cement and 0.50% biogranule (ACDC) form bacteria were used. The unit prices used in the calculation and the cost of self-healing bio-concrete obtained by microbial means are given in Table 4.20.

Table 4. 20. Cost of self-healing concrete with microbial means, which was previously stated to be developed using bacteria in biogranule for (Edited according to [111]).

Admixtures	**Material price (€/100 kg)**	**Amount used for self-healing concrete[1] (kg)**	**Bio-%0,50 cost (€/m^3 concrete)**
$Ca(HCOO)_2$	50	9	4.5
$Ca(NO_3)_2.4H_2O$	30.5	19.5	5.9
Biogranule	5740	3.2	183.7
Concrete			85
Total			279

[1]Bio-concrete contains 3.00% calcium nitrate, 2.00% calcium formate and 0.50% bacteria in biogranule form by weight of cement. Bacteria / Biogranule ratio is given as 0.7 g / g.

In this study, it has been revealed that bacteria in the form of biogranules at the rate of 0.05% by weight of cement are sufficient to obtain a cementitious composite that heals itself by microbial means. Over the unit prices given in Table 4.20, when the cost of self-

healing bio-concrete to be obtained with the mixture composition determined in this study (5.00% calcium formate, 2.00% calcium nitrate and 0.05% bacteria in the form of biogranules) is calculated, the cost of Bio-0.05% concrete is approximately 120 €/m^3. (Table 4.21). With the results obtained within the scope of this thesis, it has been revealed that the cost of bio-concrete can be reduced by 58%. Although Bio-0.05% specimens revealed better healing results than reference and control specimens and faster healing compared to specimens containing more than 0.25% biogranules, obtained crack widths in Bio-0.05% were quite limited. Therefore, Bio-0.25% specimens can be considered more promising than Bio-0.05% specimens since they revealed a wider range of crack widths and similar results. When the cost of Bio-0.25% specimens was calculated, approximately 192 €/m^3 was found. It can attribute a 32% reduction on the cost of bio-concrete.

Table 4. 21. The cost of self-healing concrete with microbial means that can be obtained with the minimum required dose of bacteria.

Admixtures	**Material price (€/100 kg)**	**Amount used for self-healing concrete[1] (kg)**	**Bio-%0,05 cost (€/m^3 concrete)**
$Ca(HCOO)_2$	50	22.5	11.3
$Ca(NO_3)_2.4H_2O$	30.5	13	3.9
Biogranule	5740	0.32	18.4
Concrete			85
Total			119

[1]Bio-concrete contains 3.00% calcium nitrate, 2.00% calcium formate and 0.05% bacteria in biogranule form by weight of cement. Bacteria / Biogranule ratio is given as 0.7 g / g.

5. CONCLUSION

Within the scope of this thesis study, microbial self-healing ability in the cement-binder system was investigated in terms of production of compatible biogranules, effects of these biogranules on fresh and hardened properties of cementitious material, and self-healing rates and capacity. Production of compatible biogranules was done in accordance with pre-determined method by thesis co-advisor's previous study and compatibility of different biogranules doses with cementitious matrix was evaluated by setting, flow and compressive strength tests. Self-healing rates and capacities for mortar specimens contains biogranules with different doses were analyzed by macroscopic inspections. The following outcomes have been obtained as a result of the experimental researches defined in the previous sections:

- Biogranules in approximately two months were successfully achieved with the use of a minimum nutrient medium that lacks micronutrients, trace elements and vitamins. The average granule size inside the reactor was 0.950±20 mm. Since if the microbial agglomerates are larger than 0.2 mm in size, they are considered biogranules and SVI_{30}/SVI_5 ratio was about 85%, it was accepted that biogranulation was done.
- The kinetic analysis shows that the sizes of biogranules are small enough for access to the core of nutrients, and big enough to prevent oxygen from propagating into the core. Furthermore, kinetic test revealed that the core of biogranules was reduced by compact nitrate as NO_x-N, while the DO levels range from 1 to 6 mg/L. In addition to the visual observations via light microscope by observing darker color outside, lighter color inside, this can be an indicator of denitrifying core.
- It is important that biogranules contain $CaCO_3$ and $Ca_3(PO_4)_2$, as well as in which layer of biogranules these minerals are formed in terms of biogranule quality. SEM combined with EDS verify the information obtained under the light microscope. Ca, P, O elements in biogranules was observed via EDS analysis.
- The chemical composition of biogranules was determined by Fourier transform infrared (FTIR) analysis. Biogranules obtained as a result of FTIR analyzes performed at different times were confirmed to contain $CaCO_3$ and $Ca_3(PO_4)_2$ as planned. The chemical composition of the biogranules that are produced

continuously and collected at different times are similar and this supports continuity of the production reactor.

- In order to investigate, effects of different biogranule doses on fresh mortar properties, samples contain biogranules from 0.25% to 3.00% w/w cement were prepared. Twp set accelerators was used as nutrients therefore a considerable reduction has become in the initial setting time. It was determined that bacteria in the form of biogranules added to the control mixture up to 2.50% by weight of cement did not cause a significant change in setting start and set ending times compared to the control sample. When this dose was increased to 3.00%, a significant decrease was observed in the starting time of setting. The setting start time of the Bio-3.00% mixture was measured as 34 ± 12 minutes, well below the acceptable 60 minutes limit. The outcome of the study, showed that integration of biogranules (with EPS and mineral layer) prevented a possible interaction between the cement matrix and the organic material, and hence no retardation on set was recorded, even at high dose bacteria (e.g. Bio-3.00%).
- The flow values of the different mixtures were compared with the flow value of the plain mortar mixture (reference). Accordingly, it was observed that even the flow of the Bio-3.00% mixture, which has the lowest flow, remained within the 10% deviation limit. Based on comparison with the flow values of the control mixture, since the flow value of the Bio-3.00% mixture corresponds to 89% of the control flow, the Bio-2.50% mixture has been determined as an acceptable upper limit. Considering that the maximum amount of bacteria in the form of biogranules that can be used in fresh mortar experiments was determined as 2.50%, it can be said that the part related to fresh mortar experiments was successfully completed without the need for any setting accelerator or super plasticizer.
- When bacteria were added in the form of biogranules, it was found that adding bacteria up to a dose of 2.00% by weight of cement did not have a statistically significant effect on 3-day compressive strength performance ($p = 0.05$). However, adding bacteria at a dose of 2.50% and 3.00% even in the form of biogranules caused a decrease of 26% in 3-day strength compared to the reference value. Although the 3-day strengths of the Bio-2.50% and Bio-3.00% samples were lower, at the end of 7 days, all biomortars reached a compression strength of around 40 MPa and did not differ statistically from each other ($p = 0.05$). Bio-

mortar samples showed a 30% better performance and reached strengths around 56 MPa on the 28th day. Except for the Bio-3.00% sample, all other mortar mixtures performed 15% to 25% better than the reference sample in terms of 56 days compressive strength.

- In order to investigate self-healing ability of bio-mortars, different doses from 0.25% to 2.50% w/w cement biogranules were used. The results revealed that the increase in the bacterial dose in the form of biogranules in the mortar mixture negatively affected the healing rate and healing capacity. The highest healing capacity was obtained in the Bio-0.25% sample with 800 ± 30 μm. In addition, the mixture that reached the healing capacity the fastest was Bio-0.25% within 3 weeks. The four-week healing capacities of the higher doses of Bio-0.50% and Bio-0.75% samples were not statistically different and were around 500 μm ($p = 0.05$). In the Bio-1.00% sample, the four-week healing capacity decreased slightly, reaching around 300 μm. It was observed that the four-week healing capacity of the Bio- 1.25% and Bio- 1.50% samples also fell below 300 μm. Four-week healing capacity decreases below 200 μm when more than 1.50% of the weight of cement is used in the form of biogranules. Specimens with an initial bacterial dose of up to 1.00% healed faster than samples containing higher doses of bacteria. It has been related with germination process of bacteria.
- Within the scope of the thesis, samples were also prepared to determine the minimum amount of bacteria in the form of biogranules required to obtain a cement-binder system that heals itself by microbial means. Therefore, in order to determine whether healing was achieved at doses lower than 0.25% biogranules w/w cement, Bio-0.05% and Bio-0.10% samples were prepared. Bio-0.05% samples showed healing over 90% in all crack widths observed within 3 weeks, such as Bio-0.25 samples. The four-week healing limit for Bio-0.05% was determined as 275 μm. In the Bio-0.10% samples, all observed cracks were closed at the end of three weeks, no statistically significant increase was observed in the crack closure percentages in the fourth week and the maximum crack width that healed after four weeks was recorded as 280 μm. However, it would be useful to examine the larger crack intervals for Bio-0.05% and Bio-0.10% in future studies in terms of determining a more accurate self-healing capacity.
- In order to calculate the cost of the bioconcrete, determining effective minimum dose of biogranules was significant. In the light of the results, the minimum

amount of biogranules required to obtain cement-binder system with microbial means was considered to be 0.05% w/w cement and, the cost of Bio-0.05% concrete was calculated as approximately 120 €/m3. However, Bio-0.25% specimens were considered more promising than Bio-0.05% specimens since they revealed a wider range of crack widths. The cost of Bio-0.25% concrete was calculated as approximately 192 €/m3 and a 32% reduction on the cost of bioconcrete. These results indicated that the durability of the concrete can be enhanced by granulated bacteria due to their ability to clog cracks by calcium carbonate precipitation which can prevent water ingress and rebar corrosion.

6. REFERENCES

[1] A. N. R. E. Pe, “ERMCO Guide to EN206:2013 1.” 2014.

[2] S. Piggin, “The properties of concrete,” *Meanjin*, vol. 65, no. 4. Pergamon, pp. 184–193, Jan. 01, 2006, doi: 10.1016/b978-0-08-009595-0.50004-5.

[3] Hiroshi Yokota, “Strategic Business Plan ISO/TC 071 ,” 2016.

[4] C. le Quéré *et al.*, “Global Carbon Budget 2016,” *Earth System Science Data*, vol. 8, no. 2, 2016, doi: 10.5194/essd-8-605-2016.

[5] National Ready Mixed Concrete Association, “Concrete CO_2 Fact Sheet,” *NRMCA Publication Number 2PCO2*, no. 2, 2012.

[6] A. Chatterjee and T. Sui, “Alternative fuels – Effects on clinker process and properties,” *Cement and Concrete Research*, vol. 123. Elsevier Ltd, Sep. 01, 2019, doi: 10.1016/j.cemconres.2019.105777.

[7] P. Friedlingstein *et al.*, “Global carbon budget 2019,” *Earth System Science Data*, vol. 11, no. 4, 2019, doi: 10.5194/essd-11-1783-2019.

[8] P. Duxson, J. L. Provis, G. C. Lukey, and J. S. J. van Deventer, “The role of inorganic polymer technology in the development of ‘green concrete,’” *Cement and Concrete Research*, vol. 37, no. 12, 2007, doi: 10.1016/j.cemconres.2007.08.018.

[9] C. Meyer, “The greening of the concrete industry,” *Cement and Concrete Composites*, vol. 31, no. 8, 2009, doi: 10.1016/j.cemconcomp.2008.12.010.

[10] D. J. M. Flower and J. G. Sanjayan, “Green house gas emissions due to concrete manufacture,” *The International Journal of Life Cycle Assessment*, vol. 12, no. 5, 2007, doi: 10.1007/s11367-007-0327-3.

[11] V. C. Li, Y. M. Lim, and Y. W. Chan, “Feasibility study of a passive smart self-healing cementitious composite,” *Composites Part B: Engineering*, vol. 29, no. 6, 1998, doi: 10.1016/S1359-8368(98)00034-1.

[12] H. A. F. Dehwah, M. Maslehuddin, and S. A. Austin, “Long-term effect of sulfate ions and associated cation type on chloride-induced reinforcement corrosion in

Portland cement concretes," *Cement and Concrete Composites*, vol. 24, no. 1, 2002, doi: 10.1016/S0958-9465(01)00023-3.

[13] B. Klemczak and A. Knoppik-Wróbel, "Early age thermal and shrinkage cracks in concrete structures - description of the problem," *Architecture Civil Engineering Environment*, vol. 4, no. 2, 2011.

[14] A.C.I.C. 224, "224R-01: Control of Cracking in Concrete Structures (Reapproved 2008)," *Technical Documents*.

[15] R.S. Narayanan, " EN1992 Eurocode 2: Design of concrete structures ," *Proceedings of the Institution of Civil Engineers - Civil Engineering*, vol. 144, no. 6, 2001, doi: 10.1680/cien.2001.144.6.23.

[16] M. Maes and N. de Belie, *Combined Effects of Chlorides and Sulphates on Cracked and Self-Healing Concrete in Marine Environments*, vol. PhD, no. NUR 955. 2015.

[17] M. Li and V. C. Li, "Cracking and healing of engineered cementitious composites under chloride environment," *ACI Materials Journal*, vol. 108, no. 3, 2011, doi: 10.14359/51682499.

[18] D. Snoeck, S. Steuperaert, K. van Tittelboom, P. Dubruel, and N. de Belie, "Visualization of water penetration in cementitious materials with superabsorbent polymers by means of neutron radiography," *Cement and Concrete Research*, vol. 42, no. 8, 2012, doi: 10.1016/j.cemconres.2012.05.005.

[19] M. Sahmaran, G. Yildirim, and T. K. Erdem, "Self-healing capability of cementitious composites incorporating different supplementary cementitious materials," *Cement and Concrete Composites*, vol. 35, no. 1, 2013, doi: 10.1016/j.cemconcomp.2012.08.013.

[20] A. Mignon *et al.*, "PH-responsive superabsorbent polymers: A pathway to self-healing of mortar," *Reactive and Functional Polymers*, vol. 93, 2015, doi: 10.1016/j.reactfunctpolym.2015.06.003.

[21] R. Davies *et al.*, "Large scale application of self-healing concrete: Design, construction, and testing," *Frontiers in Materials*, vol. 5, 2018, doi: 10.3389/fmats.2018.00051.

[22] C. Joseph, A. D. Jefferson, B. Isaacs, R. Lark, and D. Gardner, "Experimental investigation of adhesive-based self-healing of cementitious materials," *Magazine of Concrete Research*, vol. 62, no. 11, 2010, doi: 10.1680/macr.2010.62.11.831.

[23] J. Y. Wang, D. Snoeck, S. van Vlierberghe, W. Verstraete, and N. de Belie, "Application of hydrogel encapsulated carbonate precipitating bacteria for approaching a realistic self-healing in concrete," *Construction and Building Materials*, vol. 68, 2014, doi: 10.1016/j.conbuildmat.2014.06.018.

[24] J. Wang, J. Dewanckele, V. Cnudde, S. van Vlierberghe, W. Verstraete, and N. de Belie, "X-ray computed tomography proof of bacterial-based self-healing in concrete," *Cement and Concrete Composites*, vol. 53, 2014, doi: 10.1016/j.cemconcomp.2014.07.014.

[25] V. Wiktor and H. M. Jonkers, "Quantification of crack-healing in novel bacteria-based self-healing concrete," *Cement and Concrete Composites*, vol. 33, no. 7, 2011, doi: 10.1016/j.cemconcomp.2011.03.012.

[26] J. Y. Wang, H. Soens, W. Verstraete, and N. de Belie, "Self-healing concrete by use of microencapsulated bacterial spores," *Cement and Concrete Research*, vol. 56, 2014, doi: 10.1016/j.cemconres.2013.11.009.

[27] M. de Rooij, E. Schlangen, and C. Joseph, *Self-Healing Phenomena in Cement-Based Materials*, vol. 1. 2013.

[28] K. van Tittelboom and N. de Belie, "Self-healing in cementitious materials-a review," *Materials*, vol. 6, no. 6, 2013, doi: 10.3390/ma6062182.

[29] F. B. da Silva, N. de Belie, N. Boon, and W. Verstraete, "Production of non-axenic ureolytic spores for self-healing concrete applications," *Construction and Building Materials*, vol. 93, 2015, doi: 10.1016/j.conbuildmat.2015.05.049.

[30] C. Edvardsen, "Water permeability and autogenous healing of cracks in concrete," *ACI Materials Journal*, vol. 96, no. 4, 1999, doi: 10.14359/645.

[31] "Autogenous Healing of Cement Paste," *ACI Journal Proceedings*, vol. 52, no. 6, 1956, doi: 10.14359/11661.

[32] S. Joshi, S. Goyal, A. Mukherjee, and M. S. Reddy, "Microbial healing of cracks in concrete: a review," *Journal of Industrial Microbiology and Biotechnology*, vol. 44, no. 11. 2017, doi: 10.1007/s10295-017-1978-0.

[33] K. van Tittelboom, N. de Belie, D. van Loo, and P. Jacobs, "Self-healing efficiency of cementitious materials containing tubular capsules filled with healing agent," *Cement and Concrete Composites*, vol. 33, no. 4, 2011, doi: 10.1016/j.cemconcomp.2011.01.004.

[34] W. Li, Z. Jiang, Z. Yang, N. Zhao, and W. Yuan, "Self-healing efficiency of cementitious materials containing microcapsules filled with healing adhesive:

Mechanical restoration and healing process monitored by water absorption," *PLoS ONE*, vol. 8, no. 11, 2013, doi: 10.1371/journal.pone.0081616.

[35] L. Sun, W. Y. Yu, and Q. Ge, "Experimental research on the self - Healing performance of micro - Cracks in concrete bridge," in *Advanced Materials Research*, 2011, vol. 250–253, doi: 10.4028/www.scientific.net/AMR.250-253.28.

[36] Y. Yang, M. D. Lepech, E. H. Yang, and V. C. Li, "Autogenous healing of engineered cementitious composites under wet-dry cycles," *Cement and Concrete Research*, vol. 39, no. 5, 2009, doi: 10.1016/j.cemconres.2009.01.013.

[37] V. C. Li and E. N. Herbert, "Self-healing of microcracks in engineered cementitious composites (ECC) under a natural environment," *Materials*, vol. 6, no. 7, 2013, doi: 10.3390/ma6072831.

[38] Y. Kuang and J. Ou, "Self-repairing performance of concrete beams strengthened using superelastic SMA wires in combination with adhesives released from hollow fibers," *Smart Materials and Structures*, vol. 17, no. 2, 2008, doi: 10.1088/0964-1726/17/2/025020.

[39] Y. C. Kuang and J. P. Ou, "Passive smart self-repairing concrete beams by using shape memory alloy wires and fibers containing adhesives," *Journal of Central South University of Technology (English Edition)*, vol. 15, no. 3, 2008, doi: 10.1007/s11771-008-0077-9.

[40] N. de Belie *et al.*, "A Review of Self-Healing Concrete for Damage Management of Structures," *Advanced Materials Interfaces*, vol. 5, no. 17. 2018, doi: 10.1002/admi.201800074.

[41] V. Mechtcherine *et al.*, "Effect of superabsorbent polymers (SAP) on the freeze–thaw resistance of concrete: results of a RILEM interlaboratory study," *Materials and Structures/Materiaux et Constructions*, vol. 50, no. 1, 2017, doi: 10.1617/s11527-016-0868-7.

[42] A. Mignon, D. Snoeck, P. Dubruel, S. van Vlierberghe, and N. de Belie, "Crack mitigation in concrete: Superabsorbent polymers as key to success?," *Materials*, vol. 10, no. 3. 2017, doi: 10.3390/ma10030237.

[43] H. X. D. Lee, H. S. Wong, and N. R. Buenfeld, "Potential of superabsorbent polymer for self-sealing cracks in concrete," *Advances in Applied Ceramics*, vol. 109, no. 5, 2010, doi: 10.1179/174367609X459559.

[44] H. X. D. Lee, H. S. Wong, and N. R. Buenfeld, "Self-sealing of cracks in concrete using superabsorbent polymers," *Cement and Concrete Research*, vol. 79, 2016, doi: 10.1016/j.cemconres.2015.09.008.

[45] X. F. Song, J. F. Wei, and T. S. He, “A method to repair concrete leakage through cracks by synthesizing super-absorbent resin in situ,” *Construction and Building Materials*, vol. 23, no. 1, 2009, doi: 10.1016/j.conbuildmat.2007.11.009.

[46] H. M. Jonkers, “Self Healing Concrete: A Biological Approach,” in *Springer Series in Materials Science*, vol. 100, 2007.

[47] G. Nadson, “Microorganisms as Geologic Agents,” 1903.

[48] G. Nadson, “Beitrag zur Kenntnis der bakteriogenen Kalkablagerung,” *Arch Fur Hydrobiol.*, vol. 19, pp. 154–164, 1928.

[49] F. Hammes and W. Verstraete, “Key roles of pH and calcium metabolism in microbial carbonate precipitation,” *Reviews in Environmental Science and Biotechnology*, vol. 1, no. 1, 2002, doi: 10.1023/A:1015135629155.

[50] F. Hammes *et al.*, “Calcium removal from industrial wastewater by bio-catalytic CaCO3 precipitation,” *Journal of Chemical Technology and Biotechnology*, vol. 78, no. 6, 2003, doi: 10.1002/jctb.840.

[51] V. S. Whiffin, L. A. van Paassen, and M. P. Harkes, “Microbial carbonate precipitation as a soil improvement technique,” *Geomicrobiology Journal*, vol. 24, no. 5, 2007, doi: 10.1080/01490450701436505.

[52] W. de Muynck, D. Debrouwer, N. de Belie, and W. Verstraete, “Bacterial carbonate precipitation improves the durability of cementitious materials,” *Cement and Concrete Research*, vol. 38, no. 7, 2008, doi: 10.1016/j.cemconres.2008.03.005.

[53] W. de Muynck, K. Cox, N. de Belie, and W. Verstraete, “Bacterial carbonate precipitation as an alternative surface treatment for concrete,” *Construction and Building Materials*, vol. 22, no. 5, 2008, doi: 10.1016/j.conbuildmat.2006.12.011.

[54] W. R. L. van der Star, E. Taher, M. P. Harkes, M. Blauw, M. C. M. van Loosdrecht, and L. A. van Paassen, “Use of waste streams and microbes for in situ transformation of sand into sandstone,” 2010, doi: 10.3850/GI126.

[55] L. A. van Paassen, C. M. Daza, M. Staal, D. Y. Sorokin, W. van der Zon, and M. C. M. van Loosdrecht, “Potential soil reinforcement by biological denitrification,” *Ecological Engineering*, vol. 36, no. 2, 2010, doi: 10.1016/j.ecoleng.2009.03.026.

[56] G. Ganendra *et al.*, “Formate oxidation-driven calcium carbonate precipitation by Methylocystis parvus OBBP,” *Applied and Environmental Microbiology*, vol. 80, no. 15, 2014, doi: 10.1128/AEM.01349-14.

[57] F. Hammes, A. Seka, S. de Knijf, and W. Verstraete, “A novel approach to calcium removal from calcium-rich industrial wastewater,” *Water Research*, vol. 37, no. 3, 2003, doi: 10.1016/S0043-1354(02)00308-1.

[58] S. Douglas and T. J. Beveridge, “Mineral formation by bacteria in natural microbial communities,” *FEMS Microbiology Ecology*, vol. 26, no. 2. 1998, doi: 10.1016/S0168-6496(98)00027-0.

[59] N. Hearn, “Self-sealing, autogenous healing and continued hydration: What is the difference?,” *Materials and Structures/Materiaux et Constructions*, vol. 31, no. 8, 1998, doi: 10.1007/bf02481539.

[60] M. Seifan, A. K. Samani, and A. Berenjian, “Bioconcrete: next generation of self-healing concrete,” *Applied Microbiology and Biotechnology*, vol. 100, no. 6. 2016, doi: 10.1007/s00253-016-7316-z.

[61] J. W. Morse, “The kinetics of calcium carbonate dissolution and precipitation.,” *Carbonates: Mineralogy and Chemistry*, 1983.

[62] “Aquatic chemistry: chemical equilibria and rates in natural waters,” *Choice Reviews Online*, vol. 33, no. 11, 1996, doi: 10.5860/choice.33-6312.

[63] H. A. (Heinz A. Lowenstam 1912-, *On biomineralization / Heinz A. Lowenstam, Stephen Weiner*. New York: Oxford University Press, 1989.

[64] M. A. Rivadeneyra, R. Delgado, A. del Moral, M. R. Ferrer, and A. Ramos-Cormenzana, “Precipatation of calcium carbonate by Vibrio spp. from an inland saltern,” *FEMS Microbiology Ecology*, vol. 13, no. 3, 1994, doi: 10.1111/j.1574-6941.1994.tb00066.x.

[65] M. A. Rivadeneyra, J. Párraga, R. Delgado, A. Ramos-Cormenzana, and G. Delgado, “Biomineralization of carbonates by Halobacillus trueperi in solid and liquid media with different salinities,” *FEMS Microbiology Ecology*, vol. 48, no. 1, 2004, doi: 10.1016/j.femsec.2003.12.008.

[66] Y. Ersan, E. Gruyaert, G. Louis, C. Lors, N. de Belie, and N. Boon, “Self-protected nitrate reducing culture for intrinsic repair of concrete cracks,” *Frontiers in Microbiology*, vol. 6, no. NOV, 2015, doi: 10.3389/fmicb.2015.01228.

[67] Y. C. Erşan and Y. Akın, “Optimizing nutrient content of microbial self-healing concrete,” 2019.

[68] Y. Ç. Erşan, H. Verbruggen, I. de Graeve, W. Verstraete, N. de Belie, and N. Boon, “Nitrate reducing CaCO3 precipitating bacteria survive in mortar and inhibit steel

corrosion," *Cement and Concrete Research*, vol. 83, 2016, doi: 10.1016/j.cemconres.2016.01.009.

[69] B. Perito and G. Mastromei, "Molecular basis of bacterial calcium carbonate precipitation," *Progress in molecular and subcellular biology*, vol. 52, 2011, doi: 10.1007/978-3-642-21230-7_5.

[70] K. Sarayu, N. R. Iyer, and A. R. Murthy, "Exploration on the biotechnological aspect of the ureolytic bacteria for the production of the cementitious materials - A review," *Applied Biochemistry and Biotechnology*, vol. 172, no. 5. 2014, doi: 10.1007/s12010-013-0686-0.

[71] E. Tziviloglou, V. Wiktor, H. M. Jonkers, and E. Schlangen, "Bacteria-based self-healing concrete to increase liquid tightness of cracks," *Construction and Building Materials*, vol. 122, 2016, doi: 10.1016/j.conbuildmat.2016.06.080.

[72] J. Dick *et al.*, "Bio-deposition of a calcium carbonate layer on degraded limestone by Bacillus species," *Biodegradation*, vol. 17, no. 4, 2006, doi: 10.1007/s10532-005-9006-x.

[73] K. van Tittelboom, N. de Belie, W. de Muynck, and W. Verstraete, "Use of bacteria to repair cracks in concrete," *Cement and Concrete Research*, vol. 40, no. 1, 2010, doi: 10.1016/j.cemconres.2009.08.025.

[74] S. Mondal and A. (Dey) Ghosh, "Investigation into the optimal bacterial concentration for compressive strength enhancement of microbial concrete," *Construction and Building Materials*, vol. 183, 2018, doi: 10.1016/j.conbuildmat.2018.06.176.

[75] S. Joshi, S. Goyal, and M. S. Reddy, "Influence of nutrient components of media on structural properties of concrete during biocementation," *Construction and Building Materials*, vol. 158, 2018, doi: 10.1016/j.conbuildmat.2017.10.055.

[76] T. Zhu, C. Paulo, M. L. Merroun, and M. Dittrich, "Potential application of biomineralization by Synechococcus PCC8806 for concrete restoration," *Ecological Engineering*, vol. 82, 2015, doi: 10.1016/j.ecoleng.2015.05.017.

[77] H. Chen, C. Qian, and H. Huang, "Self-healing cementitious materials based on bacteria and nutrients immobilized respectively," *Construction and Building Materials*, vol. 126, 2016, doi: 10.1016/j.conbuildmat.2016.09.023.

[78] H. M. Jonkers, A. Thijssen, G. Muyzer, O. Copuroglu, and E. Schlangen, "Application of bacteria as self-healing agent for the development of sustainable concrete," *Ecological Engineering*, vol. 36, no. 2, pp. 230–235, Feb. 2010, doi: 10.1016/j.ecoleng.2008.12.036.

[79] E. Ricca and S. M. Cutting, “Emerging applications of bacterial spores in nanobiotechnology,” *Journal of Nanobiotechnology*, vol. 1. 2003, doi: 10.1186/1477-3155-1-6.

[80] J. Y. Wang, N. de Belie, and W. Verstraete, “Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete,” *Journal of Industrial Microbiology and Biotechnology*, vol. 39, no. 4, 2012, doi: 10.1007/s10295-011-1037-1.

[81] H. Jonkers, “Bacteria-based self-healing concrete,” *Heron*, vol. 56, Jan. 2011.

[82] J. Wang, K. van Tittelboom, N. de Belie, and W. Verstraete, “Use of silica gel or polyurethane immobilized bacteria for self-healing concrete,” *Construction and Building Materials*, vol. 26, no. 1, 2012, doi: 10.1016/j.conbuildmat.2011.06.054.

[83] J. Zhang *et al.*, “Immobilizing bacteria in expanded perlite for the crack self-healing in concrete,” *Construction and Building Materials*, vol. 148, pp. 610–617, Sep. 2017, doi: 10.1016/j.conbuildmat.2017.05.021.

[84] J. Y. Wang, H. Soens, W. Verstraete, and N. de Belie, “Self-healing concrete by use of microencapsulated bacterial spores,” *Cement and Concrete Research*, vol. 56, pp. 139–152, Feb. 2014, doi: 10.1016/j.cemconres.2013.11.009.

[85] J. Wang, J. Dewanckele, V. Cnudde, S. van Vlierberghe, W. Verstraete, and N. de Belie, “X-ray computed tomography proof of bacterial-based self-healing in concrete,” *Cement and Concrete Composites*, vol. 53, pp. 289–304, Oct. 2014, doi: 10.1016/j.cemconcomp.2014.07.014.

[86] D. Palin, V. Wiktor, and H. M. Jonkers, “A bacteria-based self-healing cementitious composite for application in low-temperature marine environments,” *Biomimetics*, vol. 2, no. 3, 2017, doi: 10.3390/biomimetics2030013.

[87] R. Mors and H. M. Jonkers, “Bacteria-based self-healing concrete: evaluation of full scale demonstrator projects,” *RILEM Technical Letters*, vol. 4, 2020, doi: 10.21809/rilemtechlett.2019.93.

[88] Y. Ç. Erşan, F. B. da Silva, N. Boon, W. Verstraete, and N. de Belie, “Screening of bacteria and concrete compatible protection materials,” *Construction and Building Materials*, vol. 88, 2015, doi: 10.1016/j.conbuildmat.2015.04.027.

[89] F. Silva, “Up-scaling the production of bacteria for self-healing concrete application,” 2015.

[90] H. Rong *et al.*, "Influence of bacterial concentration on crack self-healing of cement-based materials," *Construction and Building Materials*, vol. 244, 2020, doi: 10.1016/j.conbuildmat.2020.118372.

[91] F. B. Silva, N. Boon, N. de Belie, and W. Verstraete, "Industrial application of biological self-healing concrete: Challenges and economical feasibility," *Journal of Commercial Biotechnology*, vol. 21, no. 1, 2015, doi: 10.5912/jcb662.

[92] T. van Mullem, E. Gruyaert, R. Caspeele, and N. de Belie, "First large scale application with self-healing concrete in belgium: Analysis of the laboratory control tests," *Materials*, vol. 13, no. 4, 2020, doi: 10.3390/ma13040997.

[93] Y. Song *et al.*, "A novel granular sludge-based and highly corrosion-resistant bio-concrete in sewers," *Science of The Total Environment*, vol. 791, p. 148270, Oct. 2021, doi: 10.1016/j.scitotenv.2021.148270.

[94] D. J. Randall and T. K. N. Tsui, "Ammonia toxicity in fish," in *Marine Pollution Bulletin*, 2002, vol. 45, no. 1–12, doi: 10.1016/S0025-326X(02)00227-8.

[95] Y. Ç. Erşan, E. Hernandez-Sanabria, N. Boon, and N. de Belie, "Enhanced crack closure performance of microbial mortar through nitrate reduction," *Cement and Concrete Composites*, vol. 70, 2016, doi: 10.1016/j.cemconcomp.2016.04.001.

[96] D. Gao, L. Liu, H. Liang, and W. M. Wu, "Aerobic granular sludge: Characterization, mechanism of granulation and application to wastewater treatment," *Critical Reviews in Biotechnology*, vol. 31, no. 2. 2011, doi: 10.3109/07388551.2010.497961.

[97] Y. Ç. Erşan and T. H. Erguder, "The effects of aerobic/anoxic period sequence on aerobic granulation and COD/N treatment efficiency," *Bioresource Technology*, vol. 148, 2013, doi: 10.1016/j.biortech.2013.08.096.

[98] Y. Lv, C. Wan, X. Liu, Y. Zhang, D. J. Lee, and J. H. Tay, "Drying and re-cultivation of aerobic granules," *Bioresource Technology*, vol. 129, 2013, doi: 10.1016/j.biortech.2012.12.178.

[99] Y. C. Ersan *et al.*, "Volume fraction, thickness, and permeability of the sealing layer in microbial self-healing concrete containing biogranules," *Frontiers in Built Environment*, vol. 4, 2018, doi: 10.3389/fbuil.2018.00070.

[100] Y. C. Ersan, "Overlooked Strategies in Exploitation of Microorganisms in the Field of Building Materials," 2019.

[101] Y. Ç. Erşan, K. van Tittelboom, N. Boon, and N. de Belie, “Nitrite producing bacteria inhibit reinforcement bar corrosion in cementitious materials,” *Scientific Reports*, vol. 8, no. 1, 2018, doi: 10.1038/s41598-018-32463-6.

[102] P. Ghosh, S. Mandal, B. D. Chattopadhyay, and S. Pal, “Use of microorganism to improve the strength of cement mortar,” *Cement and Concrete Research*, vol. 35, no. 10, 2005, doi: 10.1016/j.cemconres.2005.03.005.

[103] N. Chahal, R. Siddique, and A. Rajor, “Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of fly ash concrete,” *Construction and Building Materials*, vol. 28, no. 1, 2012, doi: 10.1016/j.conbuildmat.2011.07.042.

[104] Z. Basaran Bundur, M. J. Kirisits, and R. D. Ferron, “Biomineralized cement-based materials: Impact of inoculating vegetative bacterial cells on hydration and strength,” *Cement and Concrete Research*, vol. 67, 2015, doi: 10.1016/j.cemconres.2014.10.002.

[105] J. Y. Wang, K. van Tittelboom, N. de Belie, and W. Verstraete, “Potential of applying bacteria to heal cracks in concrete,” 2010.

[106] Y. Ç. Erşan, N. de Belie, and N. Boon, “Microbially induced CaCO3 precipitation through denitrification: An optimization study in minimal nutrient environment,” *Biochemical Engineering Journal*, vol. 101, 2015, doi: 10.1016/j.bej.2015.05.006.

[107] M. Sonmez and Y. C. Ersan, “Production of concrete compatible biogranules for self-healing concrete applications,” *MATEC Web of Conferences*, vol. 289, 2019, doi: 10.1051/matecconf/201928901002.

[108] Y. Q. Liu, B. Moy, Y. H. Kong, and J. H. Tay, “Formation, physical characteristics and microbial community structure of aerobic granules in a pilot-scale sequencing batch reactor for real wastewater treatment,” *Enzyme and Microbial Technology*, vol. 46, no. 6, 2010, doi: 10.1016/j.enzmictec.2010.02.001.

[109] Y. Liu and J. H. Tay, “State of the art of biogranulation technology for wastewater treatment,” *Biotechnology Advances*, vol. 22, no. 7, 2004, doi: 10.1016/j.biotechadv.2004.05.001.

[110] J. J. Beun, A. Hendriks, M. C. M. van Loosdrecht, E. Morgenroth, P. A. Wilderer, and J. J. Heijnen, “Aerobic granulation in a sequencing batch reactor,” *Water Research*, vol. 33, no. 10, 1999, doi: 10.1016/S0043-1354(98)00463-1.

[111] APHA, AWWA, WEF, “Standard Methods for Examination of Water and Wastewater,” *Washington: American Public Health Association*, 2012.

[112] T. H. Ergüder and G. N. Demirer, “Investigation of granulation of a mixture of suspended anaerobic and aerobic cultures under alternating anaerobic/microaerobic/aerobic conditions,” *Process Biochemistry*, vol. 40, no. 12, 2005, doi: 10.1016/j.procbio.2005.05.005.

[113] H. G. Schlegel and C. Zaborosch, *General microbiology*. Cambridge [England]; New York, NY, USA: Cambridge University Press, 1993.

[114] Y. Q. Liu and J. H. Tay, “The competition between flocculent sludge and aerobic granules during the long-term operation period of granular sludge sequencing batch reactor,” *Environmental Technology (United Kingdom)*, vol. 33, no. 23, 2012, doi: 10.1080/09593330.2012.673011.

[115] Y. Q. Liu and J. H. Tay, “Influence of cycle time on kinetic behaviors of steady-state aerobic granules in sequencing batch reactors,” *Enzyme and Microbial Technology*, vol. 41, no. 4, 2007, doi: 10.1016/j.enzmictec.2007.04.005.

[116] P. Świątczak and A. Cydzik-Kwiatkowska, “Performance and microbial characteristics of biomass in a full-scale aerobic granular sludge wastewater treatment plant,” *Environmental Science and Pollution Research*, vol. 25, no. 2, 2018, doi: 10.1007/s11356-017-0615-9.

[117] Y. Liu, S. F. Yang, and J. H. Tay, “Improved stability of aerobic granules by selecting slow-growing nitrifying bacteria,” *Journal of Biotechnology*, vol. 108, no. 2, 2004, doi: 10.1016/j.jbiotec.2003.11.008.

[118] M. K. de Kreuk and M. C. M. van Loosdrecht, “Selection of slow growing organisms as a means for improving aerobic granular sludge stability,” *Water Science and Technology*, vol. 49, no. 11–12, 2004, doi: 10.2166/wst.2004.0792.

[119] J.-H. Tay, S. Pan, Y. He, and S. T. L. Tay, “Effect of Organic Loading Rate on Aerobic Granulation. I: Reactor Performance,” *Journal of Environmental Engineering*, vol. 130, no. 10, 2004, doi: 10.1061/(asce)0733-9372(2004)130:10(1094).

[120] B. Y. P. Moy, J. H. Tay, S. K. Toh, Y. Liu, and S. T. L. Tay, “High organic loading influences the physical characteristics of aerobic sludge granules,” *Letters in Applied Microbiology*, vol. 34, no. 6, 2002, doi: 10.1046/j.1472-765X.2002.01108.x.

[121] J. H. F. Pereboom, “Strength characterisation of microbial granules,” in *Water Science and Technology*, 1997, vol. 36, no. 6–7, doi: 10.1016/S0273-1223(97)00517-9.

[122] D. R. de Graaff, E. J. H. van Dijk, M. C. M. van Loosdrecht, and M. Pronk, "Strength characterization of full-scale aerobic granular sludge," *Environmental Technology (United Kingdom)*, vol. 41, no. 13, 2020, doi: 10.1080/09593330.2018.1543357.

[123] Y. Liu, S. F. Yang, and J. H. Tay, "Elemental compositions and characteristics of aerobic granules cultivated at different substrate N/C ratios," *Applied Microbiology and Biotechnology*, vol. 61, no. 5–6, 2003, doi: 10.1007/s00253-003-1246-2.

[124] P. N. Lens, M. P. de Poorter, C. C. Cronenberg, and W. H. Verstraete, "Sulfate reducing and methane producing bacteria in aerobic wastewater treatment systems," *Water Research*, vol. 29, no. 3, 1995, doi: 10.1016/0043-1354(94)00195-D.

[125] R. J. Zeng, R. Lemaire, Z. Yuan, and J. Keller, "Simultaneous nitrification, denitrification, and phosphorus removal in a lab-scale sequencing batch reactor," *Biotechnology and Bioengineering*, vol. 84, no. 2, 2003, doi: 10.1002/bit.10744.

[126] I. Isak *et al.*, "Quantification of polyhydroxyalkanoates in mixed and pure cultures biomass by Fourier transform infrared spectroscopy: comparison of different approaches," *Letters in Applied Microbiology*, vol. 63, no. 2, 2016, doi: 10.1111/lam.12605.

[127] A. M. Gumel, M. S. M. Annuar, and T. Heidelberg, "Biosynthesis and Characterization of Polyhydroxyalkanoates Copolymers Produced by Pseudomonas putida Bet001 Isolated from Palm Oil Mill Effluent," *PLoS ONE*, vol. 7, no. 9, 2012, doi: 10.1371/journal.pone.0045214.

[128] H. Justnes, "Calcium Nitrate as Multifunctional Concrete Admixture," *Alite Inform – International Analytical Review on Concrete, cement and Dry admixtures*, vol. 16, pp. 38–45, Mar. 2010.

[129] V. S. Ramachandran, "Concrete Admixtures Handbook 2nd Edition," *Concr Admixtures Handb, Prop, Sci, and Technol*, 1996.

[130] B. Khan and B. Baradan, "the Effect of Sugar on Setting-Time of Various Types of Cements," *Science Vision*, vol. 8, no. 1, 2002.

[131] H. M. Jonkers, A. Thijssen, G. Muyzer, O. Copuroglu, and E. Schlangen, "Application of bacteria as self-healing agent for the development of sustainable concrete," *Ecological Engineering*, vol. 36, no. 2, 2010, doi: 10.1016/j.ecoleng.2008.12.036.

[132] Z. B. Bundur, A. Amiri, Y. C. Ersan, N. Boon, and N. de Belie, “Impact of air entraining admixtures on biogenic calcium carbonate precipitation and bacterial viability,” *Cement and Concrete Research*, vol. 98, 2017, doi: 10.1016/j.cemconres.2017.04.005.

[133] H. Schreiberová, P. Bílý, J. Fládr, K. Šeps, R. Chylík, and T. Trtík, “Impact of the self-healing agent composition on material characteristics of bio-based self-healing concrete,” *Case Studies in Construction Materials*, vol. 11, 2019, doi: 10.1016/j.cscm.2019.e00250.

[134] V. Achal, X. Pan, and N. Özyurt, “Improved strength and durability of fly ash-amended concrete by microbial calcite precipitation,” *Ecological Engineering*, vol. 37, no. 4, 2011, doi: 10.1016/j.ecoleng.2010.11.009.

[135] B. Hilloulin *et al.*, “Monitoring of autogenous crack healing in cementitious materials by the nonlinear modulation of ultrasonic coda waves, 3D microscopy and X-ray microtomography,” *Construction and Building Materials*, vol. 123, 2016, doi: 10.1016/j.conbuildmat.2016.06.138.

[136] J. Wang, H. M. Jonkers, N. Boon, and N. de Belie, “Bacillus sphaericus LMG 22257 is physiologically suitable for self-healing concrete,” *Applied Microbiology and Biotechnology*, vol. 101, no. 12, 2017, doi: 10.1007/s00253-017-8260-2.

[137] Y. Erşan, “Microbial nitrate reduction induced autonomous self-healing in concrete,” 2016.