



## LJMU Research Online

**Mohammed, H, Ortoneda-Pedrola, M, Nakouti, I and Brás, A**

**Experimental characterisation of non-encapsulated bio-based concrete with self-healing capacity**

<http://researchonline.ljmu.ac.uk/id/eprint/12923/>

### Article

**Citation** (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

**Mohammed, H, Ortoneda-Pedrola, M, Nakouti, I and Brás, A (2020)  
Experimental characterisation of non-encapsulated bio-based concrete with self-healing capacity. Construction and Building Materials, 256. ISSN 0950-0618**

LJMU has developed **LJMU Research Online** for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact [researchonline@ljmu.ac.uk](mailto:researchonline@ljmu.ac.uk)

<http://researchonline.ljmu.ac.uk/>

# Experimental characterisation of non-encapsulated bio-based concrete with self-healing capacity

Hazha Mohammed<sup>1</sup>, Montserrat Ortoneda-Pedrola<sup>1</sup>, Ismini Nakouti<sup>2</sup>, Ana Bras<sup>1</sup>

<sup>1</sup>Built Environment and Sustainable Technologies (BEST) Research Institute, Liverpool John Moores University, UK

<sup>2</sup>Centre for Natural Products Discovery (CNPD), School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, UK

\*Corresponding Author: a.m.armadabras@ljmu.ac.uk

## Abstract

The main reason for early corrosion in Reinforced Concrete (RC) structures is crack formation within the concrete cover. Cracks can lead to leakage problems, allowing chloride, oxygen, water and other aggressive chemicals to enter into concrete and eventually causes corrosion of steel reinforcement. The paper shows some results of a novel bio concrete with biological self-healing agent, which added into the concrete mixture, autonomously and actively, inhibits the concrete cracks and potential premature reinforcement corrosion. Two compositions of concrete samples were prepared and casted – CEMI and CEMIII with 60% of Ground Granulated Blast Furnace Slag (GGBS) with and without non-encapsulated bio-product utilising iron respiring bacteria. The developed bio-mineral is capable of sealing cracks and blocking pores resulting in a delay of water-born ions in RC structures, acting as a diffusion barrier of oxygen to protect steel reinforcement's passivity towards corrosion. The fresh test results show that these concretes have the potential to be used in RC heavily reinforced and manually compacted sections with vibrations. The water absorption velocity has been significantly reduced with the inclusion of bio-agent in CEMI and CEMIII concretes samples, which was associated with pores sealing. Maximum water absorption via capillary tends to reduce at least 25% when bio-agent was introduced to concrete type CEMIII. Other results emphasizes the efficiency of the bio-product in CEMIII medium. The bio-agent does not decrease the compressive strength of tested concretes either for CEMI and CEM III. SEM observation shows that the crystals were well developed near the surface of the crack.

## 1. INTRODUCTION

Nowadays, developed countries already spending a huge share of their infrastructure maintenance budget on, repair and replacing of new and existing structures, (between 35% to 45% within the UK and 50% in the EU), this reflecting major deficiencies in past practise and current design and the techniques of the construction. Cracking of concrete and degradation are known to be a major problem in today's concrete structures within the UK [1].

Reinforced Concrete (RC) corrosion costs the UK £23b per year and is one of the major durability challenges leading to rust formation, cracking, spalling, delamination and degradation of structures. Corrosion is a natural process of electrochemical degradation of metals. Generally, it is due to either chloride ion ingestion from de-icing salts and marine exposure or concrete carbonation. Corrosion caused by chloride normally results in reinforced steel pitting whereas, carbonation causes a uniform corrosion in a failure of the passive layer.

Reinforcing steel corrosion mainly causing two major deterioration problems in reinforced concrete structures. Initially is reducing the reinforcement cross-sectional area that will decrease the structural element's overall

strength. This will reduce the transverse ribs size on the deformed rebar, decreases the primary bonding strength mechanism among the deformed bars and the concrete surrounding also, the concrete surrounding mechanical interlocking will be weakened. In fact, bond deterioration problems occur prior the bars ' cross-sectional area reduction becomes an issue. Both reinforcement section loss and bond deterioration are serious issues affecting the performance of reinforced concrete [2].

The second issue of deterioration is that the reinforcement corrosion produces expansive products. Eventually, corrosion expansive nature will inevitably trigger stresses higher than the tensile capacity of the concrete nearby and results in crack development. When concrete cracked, significantly reduces the bond performance as the confinement diminishes. Davis, et al. [2], noted a considerable reduction in bonding strength when cracks started to progress where after the width of the cracks becomes 1mm, the bond strength for the uncorroded sample was around 15% of the ultimate bonding strength. Cracks will further propagate, can cause spalling and delamination if not properly dealt with. Furthermore, it must be emphasized that cracks as such are not considered as reinforced concrete (RC) failure. However, the existence of cracks can decrease the durability of RC structures [3].

The most serious threats to the integrity, safety and durability of RC structures is the cracking related deterioration of cement-based materials [4]. For this service life problem caused by cracks, conventional repair methods are a common solution. Even though, conventional repair can extend RC structures ' service life, it has several drawbacks. Most of these repairs can last just 10 to 15 years; therefore, it is known that is difficult to realize durable repairs. Moreover, the cracks which are not accessible, such as cracks in underground concrete structures, are difficult to repair. In addition to these technical aspects, the repair costs are usually very high. The indirect costs are therefore typically several times higher than the structure's direct costs [3].

Besides this, cement and concrete are extremely energy-intensive, and they generate large quantities of CO<sub>2</sub> [1]. Cement manufacturing is known to be the major source of atmospheric CO<sub>2</sub>. Cement production accounts for 88% of anthropogenic CO<sub>2</sub> emissions whereas land-use shifts, with the remaining 12% primarily responsible for deforestation [5]. To achieve the sustainability targets, it is essential to reduce the environmental impact of RC structures [6]. Due to the unique properties of concrete it is hard to replace, however, more significantly concrete is a versatile material with many alternatives ways of mixture design to attain the similar material functionality [6]. Thus, there are numerous ways available to improve concrete durability and sustainability as well as reducing the environmental effects. There have been many research studies in recent years, with different technologies interested in reducing CO<sub>2</sub> concentration in the atmosphere and minimising the environmental impact of RC structures [5].

Research is therefore conducted to develop a concrete that capable of self-healing cracks, and possibly minimise the cost of maintenance and repair on key infrastructure. The use of Microbiologically Induced Calcite Precipitation (MICP) is a method for autonomous self-healing. This innovative method employs the metabolic behaviour of microorganisms and bio-mineral precursors incorporated in concrete in order to produce an inorganic compound acting as a healing-agent. This is normally calcium carbonate (CaCO<sub>3</sub>), usually in calcite form, where the microbial material can precipitate soon after forming into small cracks, and it is capable of reducing water permeation, aggressive chemicals and dissolved ions. Without the need for manual intervention, the concrete service life could be extended [7].

In accordance to Talaiekhazan, et al.,[8] and Alsharif, et al.,[5] various types of microorganisms have been introduced to the concrete such as: ureolytic strains, *Bacillus sphaericus*, *Bacillus megaterium*, *Bacillus lentus*, *Bacillus pasteurii*, *Bacillus pseudofirmus*, *Bacillus alkalinitrilicus*, *Halomonas euryhaline*, *Pseudomonas aeruginosa*, *Bacillus cohnii*, and *Bacillus amyloliquefaciens*, as natural self-healing agent to develop a new concrete named bio-concrete. According to literature review, the *Bacillus* genus family can be considered as the most common type of microorganisms used in the development of self-healing concrete.

A MICP healing process has three primary pathways: (i) aerobic metabolic conversion of calcium salts (ii) nitrate dissimilation, (iii) enzymatic urea hydrolysis and. Healing occurs in the aerobic metabolic conversion pathway due to inducing the oxidation of organic calcium salts by bacteria, such as calcium lactate or calcium acetate to calcium carbonate under good circumstances. The latter refers to optimum pH, temperature and with the presence of oxygen, water and nutrient. Carbon dioxide (CO<sub>2</sub>) and water are the byproducts of the transformation of calcium acetate (Ca (C<sub>4</sub>H<sub>6</sub>O<sub>4</sub>) to calcium carbonate (CaCO<sub>3</sub>), both compatible with concrete (Equation (1)). In addition, in the present of water and carbon dioxide, a weak carbonic acid may be formed that can contribute to the calcium hydroxide carbonation in the concrete (Equation (2)). This results in an improved form of autogenous healing, since the un-carbonated molecule is smaller than the carbonated version [7].



Nowadays, many self-healing approaches have been proposed by researchers, among them the calcium carbonate (CaCO<sub>3</sub>) generating microorganisms is the most common technology of microbial induced self-healing concrete cracks.

An experimental study performed by Chahal, et al.,[9] to determine the effect of “*Sporosarcina pasteurii*” microorganism on the rapid chloride migration and compressive strength of concrete prepared with and without fly ash. Cement was partially substituted with fly ash (FA) by three different percentages of (10%, 20% and 30%) by weight. The concrete mixes are made using three different cell concentrations (0, 10<sup>3</sup>, 10<sup>5</sup>, 10<sup>7</sup> cells/ml). After 28 incubation at room temperature, tests were conducted for rapid chloride permeability, water absorption and compressive strength. From the experimental results they observed that the addition of *S. pasteurii* (10<sup>5</sup> cells/ml) to the control concrete samples with 0% FA enhanced the compressive strength from 24MPa to 28MPa. Whereas, the 30% FA replacement the concrete increased strength from 21MPa to 24MPa. Similarly, the inclusion of FA with bacteria resulted in reduction of water absorption by four times less than normal concrete (control).

Siddique, et al.,[10], investigated the impact of *Bacillus aerius* (of concentration 10<sup>5</sup> cells/mL) on the concrete properties prepared with and without rice husk ash (RHA). Cement partially was replaced by RHA of percentages 0%, 5%, 10%, 15% and 20% by weight, prior to performing tests for abrasion resistance, porosity, compressive strength, chloride permeability and water absorption following (7, 28 and 56-days) incubation. The results indicated that the addition of *B. aerius* in RHA-concrete improved the strength of concrete at all testing ages, however, the best possible output was attained with 10% replacement of RHA and the maximum strength improvement was 36.1MPa to 40.0MPa with bacteria 9% following 28 days incubation. As well as, the inclusion of bacteria led to a reduction from 4% to 2% in water absorption, porosity, and permeability at 28 days.

Andalib, et al.,[11] examined *Bacillus megaterium* of five different cell concentrations (1×10<sup>6</sup> to 5×10<sup>6</sup>cfu/ml) were directly added into the fresh concrete mixture to achieve optimal bacterial concentration. The substantial increase in strength was observed at different ages for 3×10<sup>6</sup>cfu/ml. The compressive strength of highest grade of bio-concrete (50MPa) had enhanced by (24%) relative to lowest grade (30MPa) (12.8%). while, 10<sup>7</sup>(cfu/ml) had decreased the strength by (6%) in. SEM analysis was used to image the structural concrete samples porosity with and without bacteria of cell concentrations of 10<sup>3</sup> to 10<sup>7</sup>cfu/mL, the results of micro-structural investigations clearly demonstrated that bacterial suspension of 3×10<sup>6</sup>cfu/ml was intended to improve concrete characteristics, while the porosity of concrete samples increased with the bacterial suspension of 10<sup>7</sup>cfu/ml.

De Belie, et al.,[12] In their research, *Bacillus sphaericus* suspension spores (10<sup>9</sup>cells/mL) was encapsulated into hydrogels and then introduced into fresh mortar samples to investigate the effects on water permeability and their healing efficiency. The carbonate precipitating capacity of the hydrogel-encapsulated spores was in / on the

hydrogel matrix to form  $\text{CaCO}_3$ . After the addition of spores, the hydrogel's water absorption capacity was slightly increased, and the absorbed water would be released gradually into the surroundings during hydration of cemented materials. However, the result demonstrated that the mortar samples containing the hydrogel-encapsulated spores enhanced self-healing performance in both water permeability and crack filling, the greatest healed crack width was around 0.5mm and in average 68% reduction in water permeability. However, the healed crack width for the concrete samples made without bacteria were between 0 to 0.3mm and 15%-55% reduction in water permeability.

An experimental research by Tziviloglou, et al., [8], focused on the bacterial-based healing agent embedded into light weight aggregates (LWA) and combined with fresh mortar mixture. Alkaliphilic bacteria of the *Bacillus* genus,  $10^8$  spores/L, were incorporated into LWA (Liapor GmbH Germany, Liapor 0/4mm, and expanded clay particles,) by vacuum impregnation with yeast extract (4g/L), calcium lactate (200g/L). This study examined the influence of the bacterial healing agent when added into fresh mortar mixes and the evaluation of fluid tightness recovery after exposure and cracking to two separate healing regimes (wet-dry cycles and water immersion) through water permeability tests. The study discovered that replacing sand by LWA in mortar samples resulted in a significant reduction in compressive strength and bulk density of mortar. The presence of healing agent seemed to have an effect on workability, produced more workable (liquidly) mortar paste with higher air void content, besides, the cement hardening was also delayed by one day. As a result, the compressive and flexural strength of the bacterial prisms in the early age (3 days) was 54% and 63% respectively, which was less than the control samples. The lightweight mortar with the presence of bacteria, however, showed an improvement in crack sealing, especially in the case of wet / dry cycles, rather than continuous water immersion.

Encapsulation in coated perlite was studied by K. Paine [7] team. It is shown that this type of encapsulation leads to decreased mechanical properties. Replacing normal weight sand with LWA can reduce the strength of bio-based mortar samples by about 37%.

Although numerous studies have been conducted on the possibility of using MICP for concrete self-healing, insufficient studies have been conducted on the use of iron respiratory bacteria Fe (III) to protect steel from corrosion and optimising self-healing efficiency by taking into account the bacterial concentration required to effectively promote concrete self-healing and nutrient composition. The unique characteristic of these microorganisms is that they thrive in environments containing low dissolved oxygen (anaerobic conditions) and high ferric iron concentrations. Therefore, they are suitable candidates for RC structures. In iron respiration the terminal electron acceptor, Fe (III) is insoluble. As a novel idea, this study focused on utilising iron respiring bacteria Fe (III) respiration targeted to protect steel indirectly against corrosion, by utilising Fe (II) to reduce  $\text{O}_2$  as a promising method to address the concerns associated to the concrete corrosion problems.

Figure (1) shows  $\text{O}_2$  consumption mediated by direct microbial  $\text{O}_2$  respiration and Fe (II) indirect reduction (produced by Fe (III) respiration) protects steel against corrosion. The general corrosion equation is given. The ovals are cells that constantly respiring on the surface of the metal. Fe (II) iron formed by Fe (III) oxides respiration is a reduction shield that prevents  $\text{O}_2$  from attacking the steel surface layer [13].

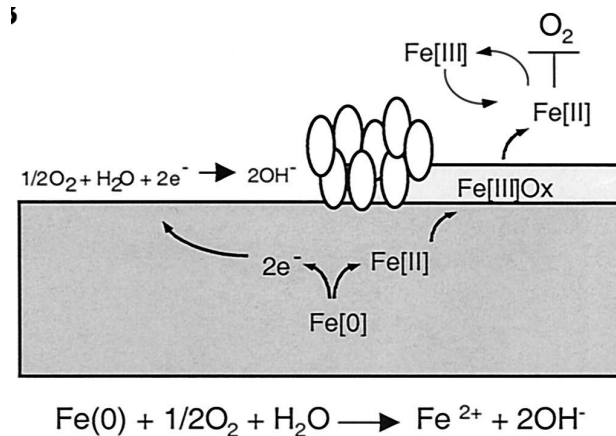


Figure 1- Schematic representation of O<sub>2</sub> consumption mediated by direct microbial O<sub>2</sub> respiration Fe (II) indirect reduction (produced by Fe (III) respiration) protects steel against corrosion (based on Dubiel et al.,[13]).

This paper is focused on biological self-healing process as a promising technique to address the concerns associated with concrete corrosion problems. Two compositions of concrete samples were prepared and casted – CEM I and CEM III with 60% of Ground Granulated Blast Furnace Slag (GGBS) with and without bio-product. Bacteria spores at different concentrations were directly added into the fresh concrete mixture to determine the best possible combinations without sacrificing concrete properties. A *Shewanella* like strain was chosen as a biological candidate as it is a facultative bacterium meaning it can grow in the presence and absence of O<sub>2</sub>. The last point is valuable as the strain is easy to handle inside and outside the concrete. It also thrives in versatile habitats and it is known to reduce a variety of metals, including iron. Bacteria spores at different concentrations were directly added into the fresh concrete mixture to determine the best possible combinations without sacrificing concrete properties. To assess the effectiveness of the self-healing concrete the following tests carried out such as, compressive strength, water absorption via capillary, open porosity test, growing bacteria evolution analysis, Scanning electron microscope (SEM).

## 2. MATERIALS AND METHODS

### 2.1 Ordinary Portland cement (CEM I)

This experimental study used type one cement (CEM I) according to BS EN 197-1, with a strength class of 52.5N. CEM I can be considered as a most widely used cement type in many different applications.

### 2.2 Ground granulated blast furnish slag (GGBS)

GGBS used for this research was sourced in the United Kingdom (Hanson Cement Limited); reported compliance with the specifications of the British Standard BS EN 15167-1 for use in mortar and concrete.

#### The Chemical Composition of CEM I and GGBS

Table 1 describes the chemical components of the components used in the preparation of concrete specimens. The products therefore have different chemical characteristics.

Table 1- Oxide percentages (%) of CEM I and GGBS [14].

Chemical Compositions	CEM I	GGBS
SiO <sub>2</sub>	19.60	54.70
Al <sub>2</sub> O <sub>3</sub>	4.71	40.87
Fe <sub>2</sub> O <sub>3</sub>	3.25	0.80
CaO	64.00	40.00
MgO	1.17	0.24
Na <sub>2</sub> O	0.27	0.20
K <sub>2</sub> O	0.73	1.95
SO <sub>3</sub>	2.94	0.00
TiO <sub>2</sub>	0.26	0.02
LOI	3.22	0.94

### 2.3 Kiln Dried Sand

The sand used in this research came from the Tarmac plant, UK. It has been tested packaged by the supplier to confirm the procedures required for quality control that comply with the British Standard BS EN ISO 9001.

### 2.4 Coarse aggregates

The aggregate used in this research sourced in UK, 10mm and 20mm aggregates used to make concrete, which they were provided, by Specialist Aggregate provided and Travis Perkins.

## 3 MICROBIAL SELF-HEALING AGENT

### 3.1 Bacterial strain and growth conditions

Bacterial strain and growth conditions *Shewanella* like strain (iron-respiring bacteria) was used in this study. The cells are usually rod-shaped Gram-negative and belong to proteobacteria (Abboud, et al., 2005). These strains are mesophilic, facultative anaerobic with respiratory metabolism, with a maximum growth temperature of 35°C. The optimum pH of the growth medium for this strain is around 7.5 (Ghosh & Mandal, 2006). The organism is Biosafety Category 1, environmentally innocuous and safe to handle.

### 3.2 Sub-culturing and growth conditions of the bio-agent:

Initially, bacterial colonies were suspended in a vial containing 20 ml of Maximum Recovery Diluent (MRD) (OXOID) and stored at 2°C (see figure 2). This culture was used when fresh inoculum was required for concrete preparation.

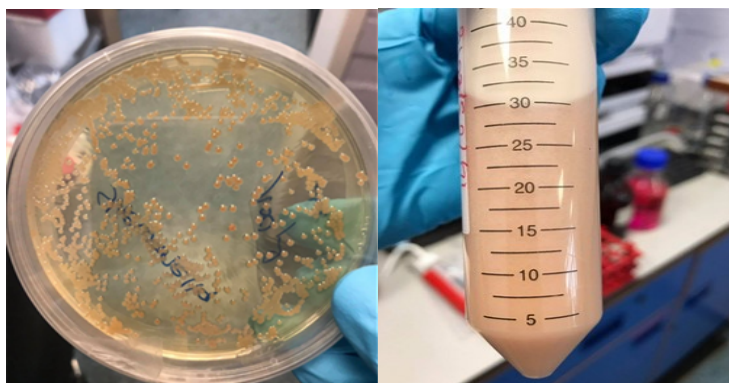


Figure 2 - Sub-culturing bio-agent.

Shake flasks (500ml) containing 125ml of sterile TSB were inoculated with 400 $\mu$ l of bacteria ( $2.3 \times 10^8$  cfu/ml) and incubated in a Benchtop Shaking Incubator for 72 hours at 30°C, 150rpm shown in figure 3.

Through serial dilution technique the growth measurement of the new culture was identified, the bio-agent concentration was  $1.7 \times 10^8$ cfu/ml, therefore, the new culture was found to be more concentrated than the original culture. Additionally, the second 500ml of bacteria cells were grown again from the same inoculum and procedure, more concentrated bio-agent was  $8 \times 10^9$  achieved. In addition, another 500ml of *Shewanella* cells were grown from different inoculum at temperature 30°C and speed 200rpm, bigger flasks were placed in Benchtop Shakier for almost around 19 hours, the highest suspension of bacteria was attained  $1 \times 10^{10}$ . In order to control the bio-agent concentration this study preferred to use the same procedure each time, furthermore, the bio-agent concentration have been regularly checked using Spectrophotometer while the flasks were still in the Benchtop Shaking Incubator. The techniques of the spectrophotometer are used for determining the concentration of solutes in solution through measuring the amount of light that the solution absorbs in a cuvette that is put in the spectrophotometer. This is the quick way to check whether the cells are growing or dying.



Figure 3 - Benchtop Shaking Incubator for growing bio-agent.

## 4 CONCRETE MIX DESIGN AND SAMPLE PREPARATION

### 4.1 Self-healing Concrete mix design

Design of a concrete mix needs to meet the performance requirements of the fresh and hardened concrete. Thus, the concrete mixture design is to optimise the concrete constituents to achieve the best possible output in terms of performance. This is done by designing two concrete mixture compositions; the first series designed and casted with 100% Ordinary Portland Cement OPC (CEMI 52.5 N), with and without bio-agent.

Bacteria was cultivated on Tryptone Soya Agar (TSA) and in shake flasks containing Tryptone Soya Broth (TSB) when liquid cultures were required.

Cultures were incubated at 30°C overnight and colony forming units were calculated by performing serial dilutions and the spread plate technique to achieve the concentration of  $2.3 \times 10^8$ cfu/ml shown in figure 4. Table 2 shows the mixture design.



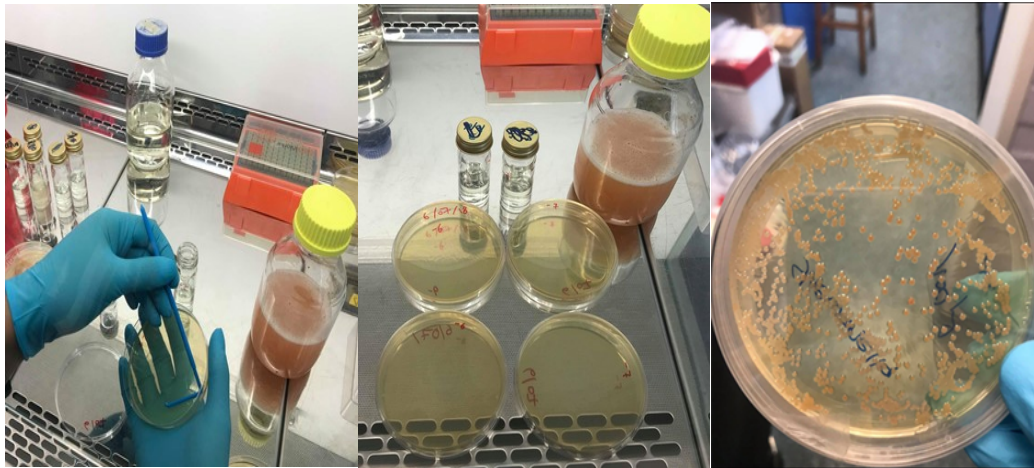


Figure 4 - Serial dilution for bio-agent.

Table 2 - CEMI concrete mix design.

Concrete Composition	kg/m <sub>3</sub>	Quantities (kg)
CEMI 52.5N	450	11.4
Agg. 20mm	712	18.1
Agg. 10mm	610	15.5
Sand	335	8.5
Water	202.5	5.1
w/b	0.45	
Bio-agent (ml)		0% and 2.1%

In the second concrete composition, 60% of OPC replaced with GGBS (CEMIII) with and without bio-agent CEMIII, table 3 shows the mixture design. The same concentration of bio-agent used in both compositions 2.1% to binder ratio. Further, the water to binder ratio w/b kept constant through the research.

Table 3 - CEMIII concrete mix design.

Concrete Composition	kg/m <sub>3</sub>	Quantities (kg)
CEMI 52.5N	180	4.6
GGBS	270	6.9
Agg. 20mm	712	18.1
Agg. 10mm	610	15.5
Sand	335	8.5
Water	202.5	5.1
w/b	0.45	
Bio-agent (ml)		0% and 2.1%

#### 4.2 Casting and curing concrete specimens.

Two series compositions of concrete samples were prepared and casted – CEMI and CEMIII with 60% of Ground GGBS with and without bio-agent. For each concrete composition three cubes of size (150 x 150 x 150mm), and six cylinders with diameter of 100mm and a height of 200mm prepared and casted (figure 5). The bacteria at different concentrations were directly introduced to the fresh concrete mixture. However, the amount of bacteria added into each concrete batch from any concentration were calculated to carry the same number of cells. After casting the mould cured under laboratory condition (20°C), the concrete samples were demoulded approximately after 24 hours, and preserved in water until the testing dates (7, 14 and 28).



Figure 5 - Concrete cubes and cylinder casting.

### 5 EXPERIMENTAL PROCEDURES AND TESTING METHODOLOGY

#### 5.1 Slump test

Slump is the most common test method utilised for determining the flow properties of fresh concrete mixture. Concrete workability is considered as a crucial property of concrete, quantified by slump test. The concrete workability can have significant effect on strength, flowability, compatibility, durability and the consistency. According to BS EN 12350-2:2009, this research study recorded slump test results for all concrete mixtures during casting.

The test was performed by filling the slump cone in three equal layers and tamping down the mixture 25 times for each layer then the cone raised upright. The difference in height among the cone and the concrete is the consistency of the slump (slump value in mm) see figure 6. The slump test is sensitive to changes in concrete consistency between 10mm and 220mm that correlates to slumps. Beyond these limits, the slump measurement may be inappropriate and other approaches should be considered for determining the consistency.



*Figure 6 - Concrete slump test.*

## **5.2 Concrete Fresh Density**

Density, it's also recognised as concrete unit weight, it is significant as part of the concrete design process since unit weight of concrete can classify the concrete categories. Some factors affecting concrete density including air content, water cement ratio w/c in the mix design and the aggregate density. Typical concrete densities are 2040kg/m<sup>3</sup> for low density concrete, 2540kg/m<sup>3</sup> for medium density concrete, and 3040 kg/m<sup>3</sup> for high density concrete [17].

The theory of Archimede's used to determine the fresh concrete density by weighing out 2kg of fresh concrete mixture and poring it into the Archimedes' can that containing water. Then the water collected from Archimedes' can that was displaced by the concrete see figure 7.



*Figure 7 - Fresh concrete density measurement.*

## **5.3 Capillary water absorption**

To determine the short term absorption capacity of each sample, a capillary test was carried out as per EN 1015-18: 2002(CEN, 2002). Samples were placed in oven at 30°C for a period of 8 days until the mass change was less than 0.1%. This test was continued until the water absorption for each sample had stabilised. The concrete cylinders with 50mm thickness were placed in 5mm of water over the absorbent paper within an airtight container to keep hydrothermal conditions consistent shown in figure 8. Weight of the samples were registered at 0', 5', 15', 30', 1h, 2h, 3h, 21h up to 28 days), until the water absorption reached to asymptotic value. It is expected that the water absorption coefficient to act as a performance characteristic for mixtures that have a direct impact on their durability.



Figure 8 - Concrete water absorption via capillary.

#### 5.4 Open porosity

Porosity test performed in compliance with BS EN 1936:2006, for concrete samples with and without bio-product in order to investigate the effect of bio-agent content. Three cylindrical samples were used in each composition with a diameter of 100mm and 50mm in length. The specimens were placed in an oven at a temperature of 30°C for 8 days to dry, then the dry mass (M<sub>1</sub>) of each sample was measured. The cylinder samples were placed in a vacuum chamber with a 30kPa vacuum pump for approximately 24 hours. On the second day, water was pumped into the vacuum chamber slowly and with the vacuum pump working for the next 24 hours. On the third day, the vacuum was released and the vacuum pump switched off; the samples remained in water in contact with atmospheric pressure for another 24 hours, as shown in Figure 9. Finally, the hydrostatic (M<sub>2</sub>) and saturated (M<sub>3</sub>) mass of each sample were measured on day four. The porosity was calculated using the following formula.

$$\text{Open porosity (\%)} = \frac{(M_3 - M_1)}{(M_3 - M_2)} \times 100$$



Figure 9 - Concrete porosity test.

### 5.5 Concrete Compressive strength.

In compliance with BS EN 12390-3:2009, the structural properties of the 150mm concrete cubes are subjected to strength testing. In a compression testing machine in compliance with EN 12390-4, samples were loaded to failure see Figure 10. The samples peak load is recorded, then the concrete compressive strength is calculated.



Figure 10 – Concrete compressive test.

### 5.6 Scanning electron microscopy (SEM)

SEM used to investigate the role of microbiologically induced mineral precipitation in healing associated with micro crack closure, enhancing the durability and strength aspects of concrete. The concrete specimens of each composition were crushed to size of 2 mm approximately and gold coated to examine healing associated with cracks closure. SEM was performed using a Scanning Electron Microscope, Fei quanta 200 esem that has the capacity to examine small cracks in high magnification.

## 6 RESULTS AND DISCUSSION

### 6.1 Slump test

Figure 11 presents the concrete slump test values recorded for each composition.

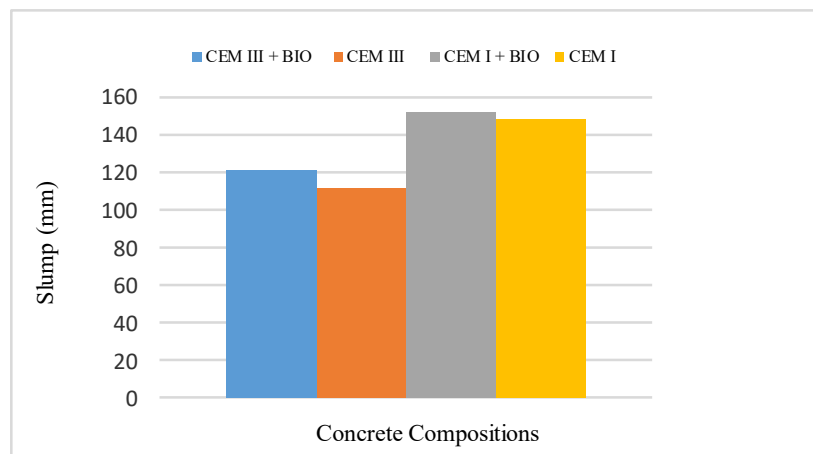


Figure 11 – Concrete slump test values recorded for each composition for w/b=0.45.

Overall, the results show that these concretes present a slump between 100 to 150mm, which corresponds to a medium workability mixes that can be either used in manually compacted flat slabs using crushed coarse aggregates, either in normal manually compacted RC and heavily reinforced units with vibrations. It is also

shown that the inclusion of bio-agent improved the workability by 10% in CEMIII particularly. However, the presence of bio-agent seemed to have no effect on CEMI.

On the other hand, the slump value of CEMIII concretes was 19% lower than for CEMI, with and without bio-agent. This reduction was attributed to GGBS high-water absorption ability. Nevertheless, the slump values for all concrete compositions were classified as S3 – Medium workability, according to BS EN 12350-2:2009.

It is highlighted here that this non-encapsulation method shows positive results from a workability perspective, increasing the facility to manufacture concrete specimens. From the opposite side, encapsulation of bacteria can lead to a decrease of workability making it enormously difficult to produce a reliable cube sample after the wall had been cast. De Belie team [12], for example, studied hydrogel encapsulated bacteria. It was found that the hydrogel water absorption capacity was increased after the inclusion of bacterial spores. Therefore, this demonstrates that the non-encapsulated bacteria can have a positive effect on workability of the cementitious materials.

## 6.2 Fresh density of concrete

Figure 12 shows the average fresh concrete densities measured for both compositions CEMI and CEMIII with and without bio-agent content.

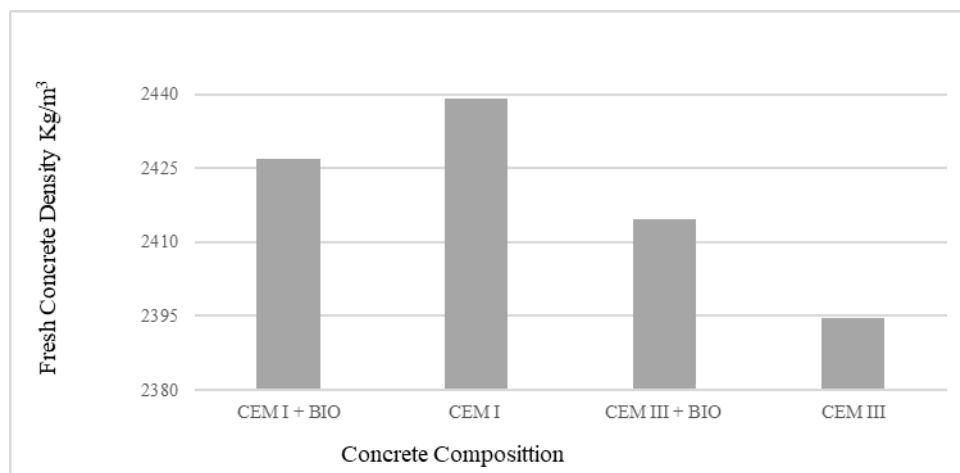


Figure 12 – Average fresh concrete density measured.

Form the results it was observed that the addition of bio-agent appears to not affect the concrete fresh density as only slightly changes were detected. Partial OPC replacement with GGBS (CEMIII) resulted in a little reduction in the concrete unit weight. The reason for this is that the specific gravity of OPC is  $3.10\text{g/cm}^3$ , which is higher than the specific gravity of GGBS which is  $2.90\text{g/cm}^3$  [18].

## 6.3 Capillary water absorption test.

Water absorption via capillary for concrete cylinders tested at 28 days is presented in the following figure (Figure 13).



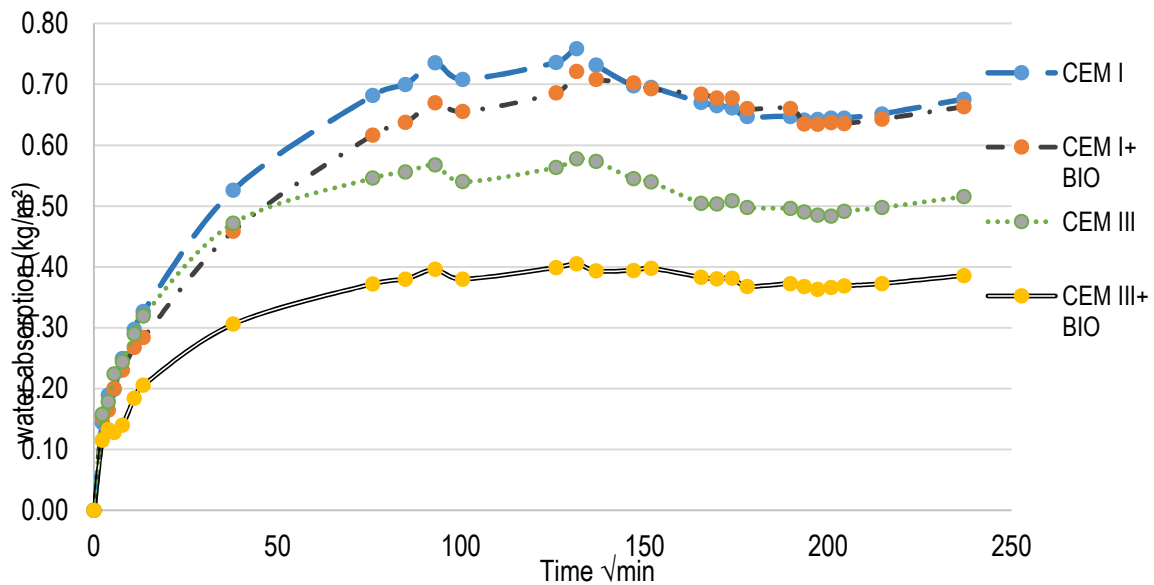


Figure 13 - Water absorption via capillary for concretes tested at 28 days and for 2 weeks.

Figure 13 demonstrates that the use of the bio-agent resulted in a reduction in water absorption slope (the angular coefficient of the curve) either for CEM I and CEM III. Beside this, the asymptotic values that correspond to the maximum absorption, is smaller for concretes with the bio-agent. Water absorption via capillary tends to reduce at least 25% when bio-agent was introduced to concrete type CEM III (CEM III+Bio). However, for CEM I concrete, the bio-agent self-healing behaviour does not change the maximum water absorbed. The analysis during the first 24 hours (figure 14) illustrates that there is a reduction in the water absorption velocity when bio-agent is introduced to CEM I and CEM III concretes.

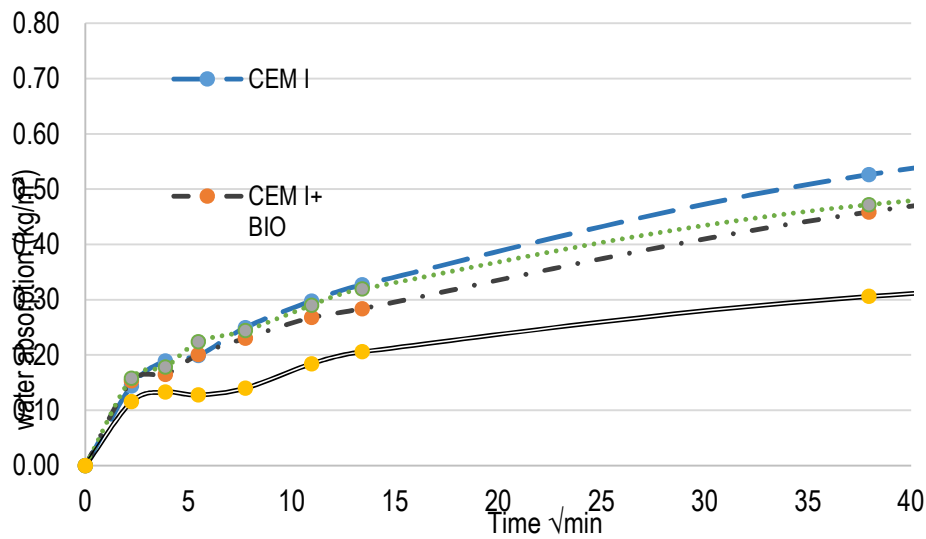


Figure 14 - Water absorption via capillary during the first 24 hours.

When considering the durability of RC and the service life it may provide, the consideration of the long-term water absorption and initial absorption velocity is imperative. Figure 13 shows the results of water absorption via capillary during the first 24 hours. It was found that there was a significant reduction in the water absorption velocity with the inclusion of bio-agent in CEM I and CEM III concretes samples, which was associated with pores sealing. Same behaviour has reported in literature by [9][10][11]. Therefore, the incorporation of self-healing agent resulted in higher resistance toward the external aggressive substances, which is associated with a service life increase of the RC structures.

The previous results also demonstrate that this bioproduct presents benefits to minimise migration of water-born ions in RC structures exposed to tidal-splash zones (water absorption in a non-saturated medium) if CEM III+bio is used. Same results are obtained for under water RC structures, which would be equivalent to the plateau obtained in the saturated part of the capillary test.

#### 6.4 Open Porosity test

Porosity of cement-based materials is a fundamental characteristic that affects physical properties, mechanical strength, service life and durability of RC structures. To understand the dynamics of water transport in the pore structure and the relationship between water and concrete, this research work carried out porosity tests for both concrete compositions CEM I and CEM III with and without bio-agent. The porosity measured for concrete cylinders tested at 28 days is shown in Figure 15. It was observed that the incorporation of bio-agent resulted in porosity reduction of 6% in CEM I and 28% CEM III after the incorporation of bio-agent.

Reduction in porosity of CEM III+Bio was more relevant than for CEM I+Bio in comparison to the plain compositions, emphasising the efficiency of the bioproduct in CEM III medium. This further reduction could be referred to replacing 60% cement to GGBS, as it has high amount iron content that could be used as nutrient for



iron respiring bacteria (*S. oneidensis*). Siddique, et al.[10] reported similar findings in literature, where the addition of bio-agent directly into the fresh concrete mixture resulted in porosity reduction.

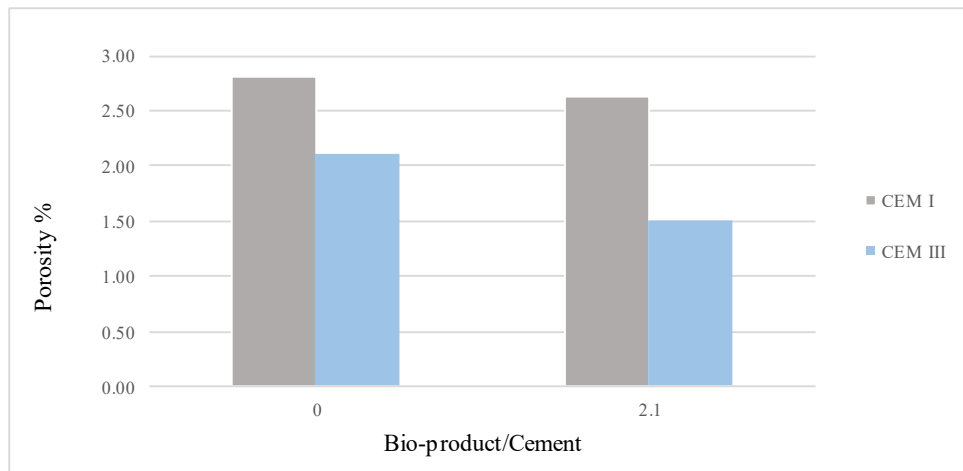


Figure 15 – Concrete open porosity test for CEM I and CEM III with and without bio-product at 28 days.

The interaction between water and the porous structure is one of the main cause RC deterioration. Due to the availability of unfilled voids and pores, concrete is known as a porous composite material. The permeability of concrete, similar to other porous materials, is characterised as the rate at which water or other liquids can ingress to concrete under a pressure head. Permeability is considered as a significant property for concrete construction, since it is negatively affecting the concrete durability that subjected to aggressive environment. Concrete permeability is not just a function of porosity, it depends on pore size, distribution, porosity and tortuosity of concrete pore channels [19].

Inevitable microcracking of the surface can result in permeability increase of the RC, make it more vulnerable to aggressive agents (atmospheric carbon dioxide CO<sub>2</sub>, water, dissolved chlorides in water) and therefore affects the long-term durability [2]. Therefore, the self-healing concrete innovative technology might allow the repair of the open micro-cracks in concrete that can threaten the integrity of structural durability, due to access of aggressive liquids and gasses. The principle of using iron respiring bacteria (*S. oneidensis*) Fe (III) respiration is aimed at protecting steel indirectly from corrosion, through using Fe (II) to minimise O<sub>2</sub>, as a promising technique to address the concerns related to concrete corrosion problems.

The laboratory experimental results proved that the bacteria (*S. oneidensis*) treated concrete samples, had lower values of porosity when compared to control samples. This demonstrates that longer time needed for water to rise through capillary actions in bio-concrete and therefore it has been revealed that the bio-concrete was less porous when compared to the control concrete samples, the principal justification for this is calcite mineral precipitation in concrete pores due to the bacterial activity.

### 6.5 Concrete compressive strength

The following figure present the average compressive strength results for concrete type CEM I and CEM III with and without bio-product – 28 days.

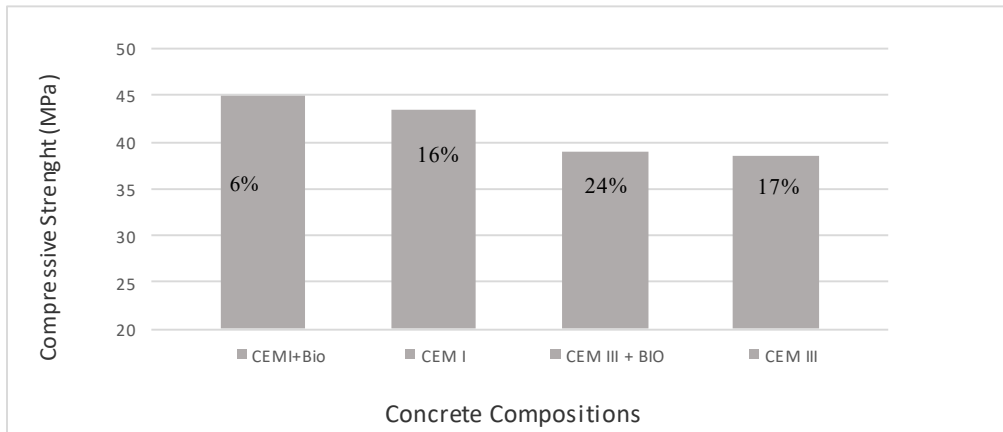


Figure 16 – Average compressive strength and coefficient of variation of concretes tested at 28 days.

The previous results show that the addition of the bio-agent does not reduce the concrete compressive strength either for CEM I and CEM III. However, for CEM I it is detected an improvement by 5% of the strength with the use of the bio-agent. Similar outcomes were reported in literature [9][10][11], where the direct addition of bio-agent into the fresh concrete/mortar mixtures had contributed in improving compressive strength. Whereas, the use of light weigh aggregates (LWA) impregnated bacteria caused a considerable reduction in mortar compressive strength, also, delayed the cement hardening around one day reported by [20].

### 6.6 Scanning Electron Microscope (SEM)

The precipitations produced at the cracks surface of the cement-based materials were analysed through SEM. The results presented in figure 17 shows CEMIII concrete with (a) and without (b) bio-agent. It is shown a clear precipitation by bacterial action remediated the micro cracks within the concrete samples CEMIII. There was no precipitation observed in the control samples as shown in figure 17b. On closer observation of CEMIII concrete with bio-agent (figure 18) shows the traces of bacterial activity present on crystal surfaces formed in the cracks of bacterial samples that were subjected to continuous water immersion for 28 days.

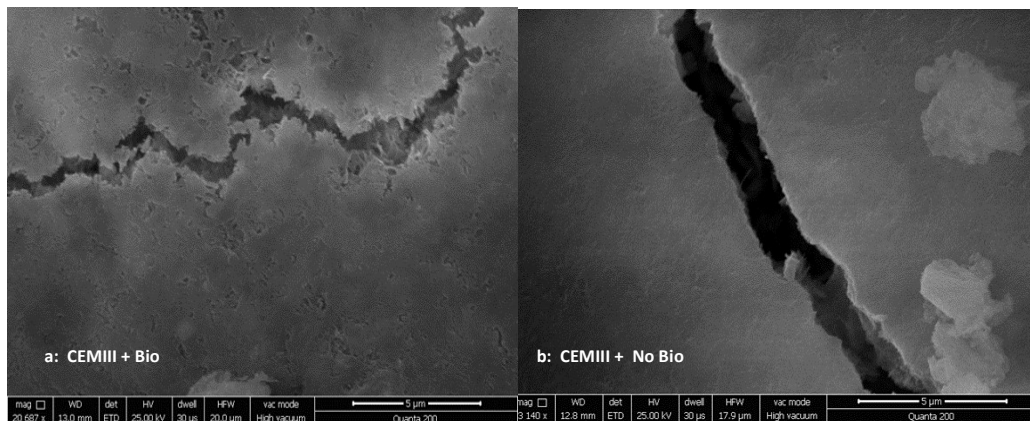


Figure 17 - SEM analysis for concrete CEMIII with (17a) and without (17b) bio product.

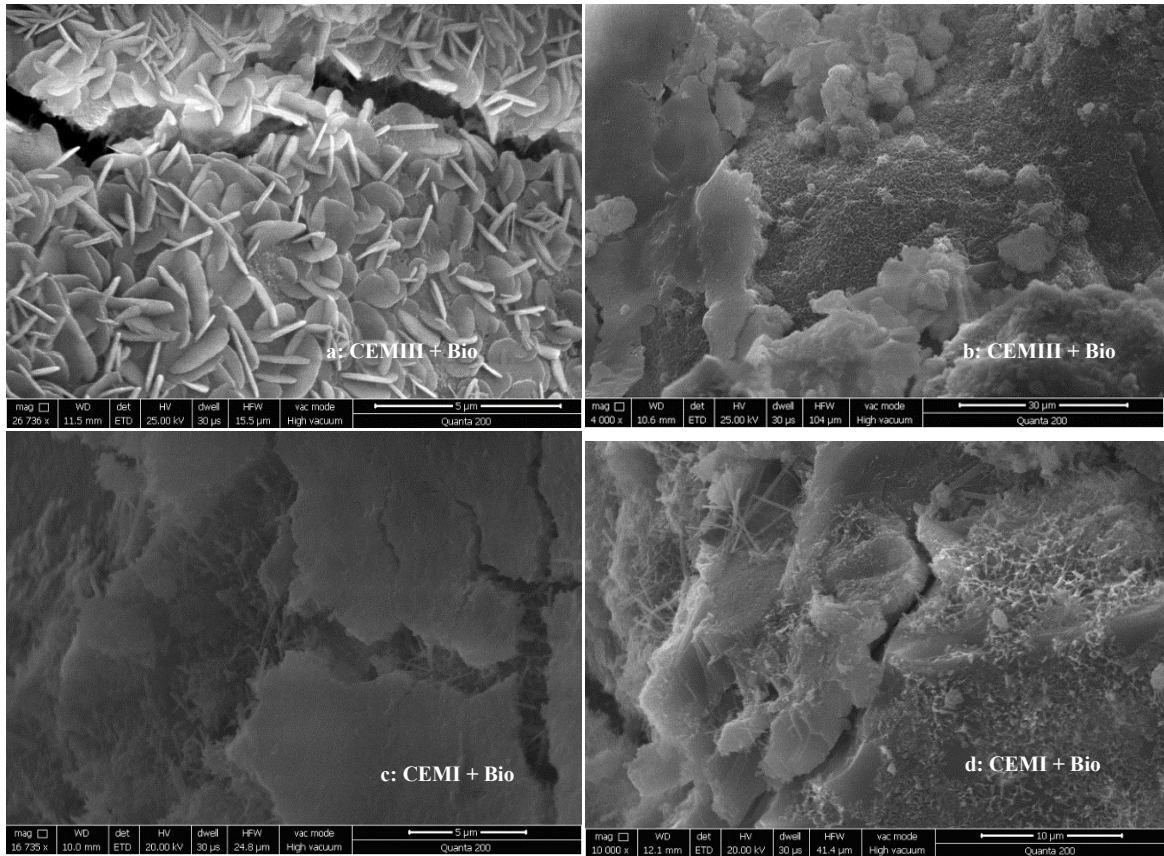


Figure 18 - Analysis of the microstructure via the SEM at 28 days for CEMIII and CEMI with bio-agent.

Figure 18 shows evidence of the development of biofilms. On the cracks remediated in the specimens containing the bacterial cells, a clear precipitation was observed. Upon closer inspection, the crystals are found to be well developed near the crack surface.

The significant role of bio-agent induced in cement-based materials is demonstrated. When oxygen and water enter the cracks the dormant spores possibly will be activated. Then, a series of biochemical reactions will be produced, and a self-healing will be obtained.

## 7. CONCLUSION

The previous results demonstrated that incorporating bacteria with fresh concrete during casting (mixing process) allows this method to be a promising technique to increase the performance of RC. Therefore, it is expected that the proposed application of sustainable biological self-healing agent will substantially decrease the maintenance and repair costs of RC structures. The potential of self-healing concrete through bio-mineralization processes in which bacteria influences the mineral precipitation is promising. In order to integrate a microorganism into cement-based materials, the primary challenges of this study were to find a suitable microorganism (bacteria) that can withstand the high alkaline environments, stay viable with minimal access to nutrients, and survive during mixing process.

Slump test showed that the addition of bio-agent increased workability up to 10% in CEMIII particularly and does not change in CEMI. The addition of bio-agent appears to not affect the concrete fresh density as only slightly changes were detected. Water absorption via capillary tends to reduce at least 25% when bio-agent was

introduced to concrete type CEMIII. However, for CEM I concrete, the self-healing behaviour does not change the maximum water absorbed. The first 24h shows a considerable reduction in water absorption velocity if self-healing is used in CEM I and CEMIII concretes, which is associated with pores sealing. Beside this, the incorporation of bio-agent resulted in porosity reduction of concrete compositions at least 6% in CEM I and 28% in CEMIII and improving compressive strength for CEM I by 5% at 28 days of age while, having no negative effect in CEMIII. The porosity reduction and improve in mechanical strength regains confirmed by the bio-concrete, can lead into durability and service life improvement. From the SEM analysis, it was found that the crystals precipitated by bacteria action filled the micro cracks within the concrete samples.

As a result, the experimental outcomes were very promising based on the available information and presented results, the novel method of the bio-based self-healing system seems to be a promising method to address the concerns associated with concrete corrosion problems. Further studies should encompass longer exposure of samples to better characterise long term performance of this type of self-healing concrete.

### Acknowledgment

The authors of this paper would like to take the Royal Society research grant RGS\R1\191304 - Research Grants 2019 Round 1 and the grant 272002/ S0001/ 911BUE that enabled the development of this work.

### REFERENCES

- [1] Gardner, D., Lark, R., Jefferson, T. & Davies, R., 2018. A survey on problems encountered in current concrete construction and the potential benefits of self-healing cementitious materials. *Case Studies in Construction Materials*, 8 February, Volume 8, pp. 238-247.
- [2] Davis, M., Hoult, N. A. & Scott, A., 2016. Distributed strain sensing to determine the impact of corrosion on bond performance in reinforced concrete. *Construction and Building Materials*, July, Volume 114, pp. 481-491.
- [3] Huang, H., 2014. *Thermodynamics of Autogenous Self-healing in Cementitious Materials*, Delft: Delft University of Technology.
- [4] Lv, L., Yang, z., Zhu, G., Han, N., Schlangen, E., Xing, F., 2016. Synthesis and characterization of a new polymeric microcapsule and feasibility investigation in self-healing cementitious materials. *Construction and Building Materials*, 15 February, Volume 105, pp. 487-495.
- [5] Alshalif, A. F. Irwan, J.M., Othman, N., Anneza, L. H., 2017. *Carbon Dioxide (CO<sub>2</sub>) Sequestration In Bio-Concrete, An Overview*. Johor, Malaysia, EDP Sciences, p. 8.
- [6] Al-Ayish, N., 2017. *Environmental Impact of Concrete Structures – with Focus on Durability and Resource Efficiency*, Stockholm: KTH.
- [7] Alazhari, M., Sharma, T. Heath, A., Cooper, R., Paine, K., 2018. Application of expanded perlite encapsulated bacteria and growth media for self-healing concrete. *Construction and Building Materials*, Volume 160, pp. 610-618.
- [8] Talaiekhazan, A., Keyvanfar, A., Shafaghat, A., Andalib, R., Abd Majid, M. Z., Fulazzakay, M. A., Zin. R. M., Lee, C. T., Hussaim, M. W., Hamzah, N., Marwar, N. F., Haidar, H.I., 2014. A Review of Self-healing Concrete Research Development. *Journal of Environmental Treatment Techniques*, 26 February, 2(1), pp. 1-11.

- [9] Chahal, N. C., Rajor, A. & Siddique, R., 2012. Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of fly ash concrete. *Construction and Building Materials*, Volume 28, pp. 351-356.
- [10] Siddique, R., Singh, K., Kunal, Singh, M., Corinaldesi, V., Rajor, A., 2016. Properties of bacterial rice husk ash concrete. *Construction and Building Materials*, Volume 121, pp. 112-119.
- [11] Andalib R, Majid M Z A, Hussin M W, Ponraj M, Keyvanfar A, Mirza J, Lee H S., 2016. Optimum concentration of *Bacillus megaterium* for strengthening structural concrete. *Construction and Building Materials*, Volume 118, pp. 180-193.
- [12] Wang J Y., Snoeck D., Van Vlierberghe S., Verstraete W., 2014. Application of hydrogel encapsulated carbonate precipitating bacteria. *Construction and Building Materials*, Volume 68, pp. 110-119.
- [13] Dubiel M, Hsu CH, Chien CC, Mansfeld F, Newman DK., 2002. Microbial Iron Respiration Can Protect Steel from Corrosion. *Appl Environ Microbiol*, 68(3), p. 1440–1445.
- [14] Sadique, M., Coakley, E, 2016. The influence of physico-chemical properties of fly ash and CKD on strength generation of high-volume fly ash concrete. *Advances in Cement Research*, 24 August , 28(9), p. 95–605.
- [15] R. Abboud, R., Popa, V., Souza-Egipsy, C. S., Giometti, S., Tollaksen, J. J., Mosher, R. H., Findlay and K. H. Nealson, 2005. Low-Temperature Growth of *Shewanella oneidensis* MR-1. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY*, February, 71(2), p. 811–816.
- [16] Ghosh, P. & Mandal, S., 2006. Development of bioconcrete material using an enrichment culture of novel thermophilic anaerobic bacteria. *Indian Journal of Experimental Biology*, 44(4), pp. 336-339.
- [17] Bai, Q. & Bai, Y., 2014. On-Bottom Stability. In: *Subsea Pipeline Design, Analysis, and Installation*. s.l.:ScienceDirect, pp. 319-335.
- [18] Mo, K. H., Alengaram, U. J. & Jumaat, Z., 2014. Utilization of ground granulated blast furnace slag as partial cement replacement in lightweight oil palm shell concrete. *Materials and Structures*, 14 May, 48(8), p. 2545–2556.
- [19] Srinivasa, V. R., Sreenivasa, d. R., Seshagiri, V. R. M. & Sasikala, C., 2013. Permeation Properties of Bacterial Concrete. *Journal of Mechanical and Civil Engineering (IOSR-JMCE)*, 5(6), pp. 08-16.
- [20] Tziviloglou, E., Wiktor, V., Jonkers, H. M. & Schlangen, E., 2016. Bacteria-based self-healing concrete to increase liquid tightness of cracks. *Construction and Building Materials*, Volume 122, pp. 118-125.