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Immobilization of *Bacillus sphaericus* with Palm Oil Fuel Ash for Self-healing in the Mortar

Husnul Azan Tajarudin¹, Rangunathan Santiago^{2,3}, Nair Gomezh⁴, Zawawi Daud⁵, Megat Azmi Megat Johari⁶, Badorul Hisham Abu Bakar⁶, Rawahah Wahdah Djuanda¹

¹School of Industrial Technology, Universiti Sains Malaysia, 11800 Pulau Pinang, Malaysia

²School of Environmental Engineering, Universiti Malaysia Perlis, Komplek Pusat Pengajian Jejawi 3, 02600, Jejawi, Perlis, Malaysia

³Sustainable Environment Research Group (SERG), Center of Excellence Geopolymer and Green Technology, School of Material Engineering, Universiti Malaysia Perlis, Kompleks Pengajian Jejawi 2, 02600 Arau, Perlis, Malaysia

⁴Centre for Research & Innovation, UOW Malaysia KDU Penang University College, 32, Jalan Anson 10400 Penang, Malaysia

⁵Solid Waste Management and Resource Recovery Team, Micropollutant Research Centre, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat, Johor, Malaysia

⁶School of Civil Engineering, Engineering Campus, Universiti Sains Malaysia, 14300 Nibong Tebal, Pulau Pinang, Malaysia

E-mail: azan@usm.edu.my

Abstract. Crack in concrete or mortar provides preferential accesses for permeation of liquids and gasses which results in degradation of concrete or mortar and structural failure. Basically, this study has used immobilized *Bacillus sphaericus* (*B.sphaericus*) with palm oil fuel ash as self-healing agent in the mortar. The strength of the mortar was improved through precipitation of calcium carbonate by *B.sphaericus* causing stronger structure due to the binding effect of calcium silicate (C-S-H) gel produced from the pozzolanic reaction. Both self-healing agents were most likely increased the performance of self-healing in the mortar. In the first phase of this study, the immobilization of both self-healing agents was studied. This was then followed by embedment of immobilized *B.sphaericus* with POFA into mortar mixture and the effect of different mortar proportion on flow table diameter, compressive strength, microstructural and atomic percentage. It was found that samples containing bio-based self-healing agent recorded the highest compressive strength of 20.95 MPa. The micrograph of SEM and EDX analysis showed that self-healing occurred when secondary C-S-H gel and calcium carbonate present in particular sample. From the result obtained, embedment of the immobilized *B.sphaericus* with POFA into mortar mixture could solve the crack issue.



1. Introduction

1.1. Self-healing Concept

Self-healing is the ability to heal by itself by recovering strength from damage such as micro crack which consists of materials that are specifically designed to act as an effective crack healing preferably during total constructions lifetime and over the course of its services life. The self-healing could be autogenomic healing, biological healing or chemical self-healing. In this study, focus is given to only two types of self-healing agents, namely autogenomic and biological healing. Autogenomic healing is the healing mechanism done via both autogenous and autonomic healing; autogenous healing is self-healing process of crack in concrete which occurs in the presence of moisture and absence of tensile stress, while autonomic healing is damage recovery process in concrete using healing agents in the concrete such as epoxy resins, cyanoacrylates (super glues), alkali-silica solutions, methyl methacrylate, expansive minerals, hydrogel and bacteria-based microorganisms. Autogenomic healing can be performed by mineral intervention such as adding fly ash such as palm oil fuel ash into concrete or mortar. Biological healing is self-healing mechanism using microbiological organism such as fungi, bacteria or viruses as self-healing agent considered as autonomic healing. The microbial activities of these agents could enhance the formation of minerals which are known as biomineralization. Figure 1 summarizes the self-healing concept.

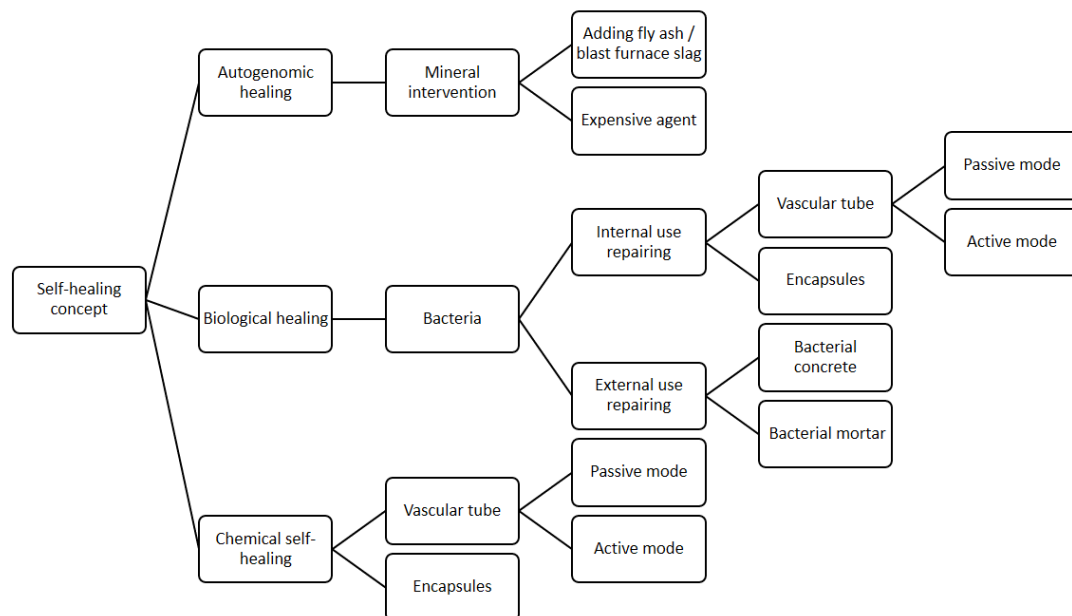


Figure 1. Taxonomy of self-healing concept.

Malaysia is considered as one of the largest palm oil exporters around the world besides Indonesia. As a result, around 4 million tonnes of palm oil flue ash (POFA) have been generated annually [1]. In palm oil production process, POFA is produced by burning palm oil fiber, shells, empty fruit bunch in the boiler with temperature of about 900°C – 1000°C [2]. Several researches have been carried out to prevent the substantial amount of POFA produced. Disposal of POFA in open dumping site continuously may affect the environment through pollution of ground water source and unsightly view. In fact, POFA has low

density thus it is easy to be blown by the wind and spread to other places causing air pollution [1]. Previous investigation was done to determine the potential of POFA as fertilizer for agricultural purposes. However, due to the absence of sufficient nutrient required for fertilizer, POFA remained to be dumped in open field without any profitable return [3]. Then, researchers tried to explore the potential of palm oil industry by-products to be used in construction industry. As a result, POFA was found to have potential as supplementary cementations material in concrete and mortar through by partial substitution of cement with POFA [4]. This is an alternative approach to reduce carbon dioxide (CO₂) emission and conserve natural energy sources [5, 6]. Normally, POFA is grey in color and becomes darker when the proportions of unburnt carbon are increasing. POFA could withstand high temperature and fill in the micro pore or crack in concrete successfully [7].

Addition of POFA with mortar could enhance the mortar strength. The chemical compositions of POFA are influenced by various factors such as burning temperature, particle fineness and parts of the palm oil tree that was burnt. Kernel burning results in high silica content whereby the durability and strength of mortar containing POFA are directly proportional to the amount of reactive silica present in the POFA. However, the development of concrete strength will be in vain if palm oil tree branches present in the burning process as more carbons are generated [8]. The strength of mortar containing POFA is attributed to the pozzolanic reaction via hydration process of calcium hydroxide (Ca(OH)₂) with silica content in POFA forming calcium silicate hydrate (C-S-H) gel as shown by equations 1, 2 and 3 [9,10,11]. C-S-H gel has high specific surface and high binding capacity, which are responsible for cohesive properties of Portland-based cements [12,13].



The capability of biomineralization process depends on types of bacteria. *B.sphaericus* is an aerobic bacterium with rod shape and it is endospore forming bacterium which is widely distributed in soil and water habitat [14]. *B.sphaericus* was used for years as a microbial pesticide to reduce mosquito vector populations [15]. Previously, *Bacillus pasteurii* is the only species that is well known to facilitate precipitation of calcium carbonate (CaCO₃). This was proven by Arunachalam *et al.*[16] when the amount of CaCO₃ precipitated was found through EDTA titration. Moreover, the selection of *B.sphaericus* incorporated into bio-concrete was due to its survival capability in high alkaline environment [17]. The optimum growth of *B.sphaericus* in pH range of 8-9 [18,19].

Biomineralization is the formation of mineral crystal by precipitation. Biological induced mineralization is one of the mechanisms involved in mineral precipitation. The biologically induced mineralization is the chemical adjusted for an environment viabiological activity resulting in precipitation of mineral crystals [20]. For example, microbial induced calcite precipitation involving microorganism [21,22] which is able to produce metabolic products such as carbonate ion (CO₃²⁻). Precipitation of CaCO₃ will occur from reaction of carbonate ion and calcium ions (Ca²⁺) from the environment. The formation of minerals occurs through common microbial metabolic activities[23,24]. One of the activities is urea hydrolysis or ureolysis which produces mineral crystals from the organism involved in the nitrogen cycle. *B.sphaericus* is an ureolytic bacteria involved in ureolysis process. Initially, the ammonia and carbonic acid are formed by hydrolysing the urea. Then, these products will be hydrolysed to form carbonate ion and hydrogen ion which increase the pH level. Since the bacteria cell wall is negatively charged, it will draw the cations from the environment including Ca²⁺ and react with CO₃²⁻ ions leading to precipitation of CaCO₃ [25]. The overall ureolysis process is simplified in equations 4, 5, 6 and 7, and figure 2.

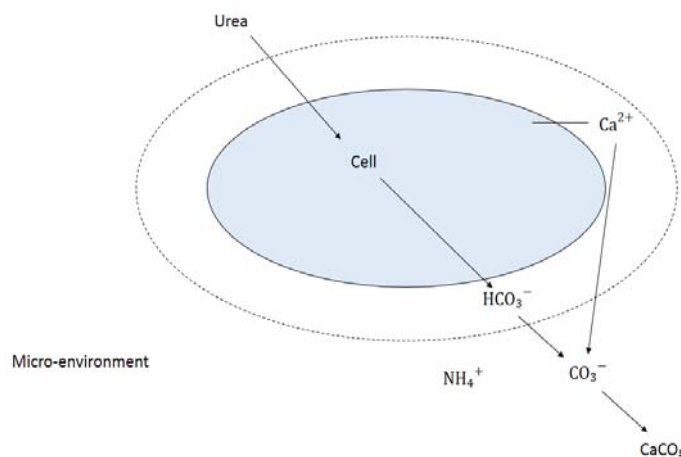
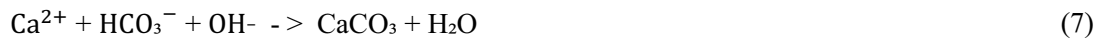


Figure 2. Schematic diagram of CaCO_3 precipitation process from ureolytic bacteria.

Immobilization of microorganism refers to the technique of restricting or anchoring the microorganism in or on inert support materials for their stability, protection and functional reuse. There are several types of immobilization methods, namely entrapment and surface immobilization. The entrapment method is where the microorganism, cells or enzyme is trapped within the space. There are three major methods of entrapment which are matrix entrapment, membrane entrapment and microencapsulation entrapment using polymeric material such as Ca-alginate, agar, k-carrageenin, polyacrylamide and collagen [26]. Surface immobilization uses adsorption and covalent bonding to immobilize the microorganism, cells or enzyme on the surface of support materials. Apart from that, adsorption is the interaction of cells with the surface of support particles to attach each other by forces such as van der Waals of dispersion which is a weak physical force. However, desorption of cells is a common problem since binding forces are weak. Meanwhile, covalent bonding is the attachment of the cells on the support surface by covalent bond [27]. Microorganism, cells or enzyme will bind to the support surface due to certain functional groups such as carboxyl, amino, sulfhydryl and hydroxyl group. In this study, immobilization of *Bacillus sphaericus* is necessary due to the harsh environment within the concrete matrix that can damage the bacterial cells [28]. Palm oil fuel ash is able to be used as support material due to the very porous and spongy surface [8]. It is also able to sustain the viability of *B.sphaericus* for long period of time and assist them to withstand the mechanical forces during casting of concrete or mortar [28,29].

Mortar is used to hold together the building materials such as brick or stone. It is composed of a thick mixture of water, sand and cement. The water is used to hydrate the cement and hold the mix together. The water to cement ratio is higher in mortar than in concrete in order to form its bonding element. When mixed, mortar forms a much thicker substance than concrete, making it ideal as a glue for building materials like brick or concrete. The production of building materials will keep increasing as more

sidewalks, buildings, and bridges are built the urban areas. Strength of mortar is gradually enhanced and improved for compressive load resistance. However, if the load applied exceeds the limit, crack will occur resulting in strength reduction. Thus, researchers have come out with several solutions to reduce maintenance cost and repair the crack by autogenous healing, encapsulation of polymeric materials and microbial production of calcium carbonate [29, 30]. One of the strategies to repair the crack is by incorporating bacteria in concrete with the ability to produce calcium carbonate crystal which blocks the micro cracks and pores in the concrete [19, 31]. Calcium carbonate precipitates on the surface of cells and ultimately within the pores which result in sealing and blockage of oxygen and nutrients flow into the cells. The cells either die or turn into endospore and act as organic fibers that contribute to the improvement of compressive strength of cement mortar [32]. Some researchers suggested adding some minerals additives such as palm oil fuel ash (POFA) into concrete when mixing. When cracking occurs, water from the environment can penetrate into the cracks and activate physical-chemical reaction and produce C-S-H gel that will close the cracks [31, 33,34]. Other than that, C-S-H gel creates a stronger bond between paste and other aggregates resulting higher strength of mortar[1, 35].Therefore, the aim of this study is to immobilize *Bacillus sphaericus* with palm oil fuel ash (POFA) at optimum condition and investigate the difference between normal mortar, mortar mixed with POFA and mortar mixed with POFA and *Bacillus sphaericus* in terms of flow table diameter, compressive strength and atomic percentage. The morphologies and elements present in the normal mortar, mortar mixed with POFA and mortar mixed with POFA and *Bacillus sphaericus* are also studied.

2. Experimental methods

2.1. Preparation of Inoculum (*Bacillus sphaericus*)

Bacillus sphaericus LMG 225577 (Belgian Co-coordinated Collections of Micro-organisms) were used in this study. *B.sphaericus* was cultured in urea yeast extract medium. This medium was prepared by mixing the autoclaved of 20 g/L of urea and 20 g/L of yeast extract separately for 20 minutes at 120°C. The culture then was allowed to grow for 24 hours in an orbital shaker with 200 rpm at 30°C. Next, the bacterial cells were harvested via centrifugation (5000rpm, 7 min). The cell pellets were washed several times to wash out the medium. The cells were preserved in saline solution (NaCl, 8.5 g/L) and then stored in fridge at 4°C [36]. The concentration of bacterial cells in the suspension was 10^9 cells/ml.

2.2. Preparation of Palm Oil Fuel Ash (POFA)

Prior to conducting any experiment, POFA was obtained from the local palm oil mill and oven dried at $110 \pm 5^\circ\text{C}$ for 24 hours to remove moisture. Then, it was sieved using the 300- μm sieve to remove large unwanted particles. The POFA was grounded using the ball mill until the median particle size was reduced to about 10 μm [3]. Then, it was heated at 450°C for 1.5 hours to remove unburned carbon [10]. All the preparation processes of POFA were carried out at School of Civil Engineering, Universiti Sains Malaysia. The prepared POFA was then oven dried to remove the moisture. Prior to sterilization, the prepared POFA was autoclaved for 20 minutes at 120°C. Characterization of POFA was performed using Brunauer-Emmet-Teller (BET) surface area analysis to access the specific particle size and pore size distribution. All the values were obtained from adsorption of nitrogen gas (N_2) at 77 K using Autosorb I, supplied by Quantachrome Corporation, USA. Prior to measurements, the sample was outgassed under vacuum at 200°C for 3 hours[37].

2.3. Optimization of Immobilization

The immobilization steps were optimized in order to obtain optimum concentration and condition for immobilization of *B.sphaericus* with POFA. 10^9 Cell/ml bacterial suspension in saline solution was used

in this procedure. Different concentration of POFA (w/v), *i.e.* 2g POFA + 10 ml BS (20%), 4g POFA + 10 ml BS (40%), and 5g POFA + 10 ml BS (50%) were used in the pre-mixture and all of them were put on shaker for 1 hour at temperature of 30°C and different rotation rate (70rpm and 100rpm). The detailed information of experimental arrangement can be seen in table 1. The attachment of the bacteria with POFA was then analyzed using Fourier Transform Infrared (FTIR) analysis. To ensure the viability of the bacteria in the POFA mixture, the immobilized sample was dried in the desiccator and cultivated on urea yeast extract agar medium.

Table 1. POFA concentration in bacterial suspension with different orbital shaker rotation rate.

Sample of POFA	POFA Concentration in Bacterial Suspension		
	POFA (g)	Bacterial Suspension (ml)	Revolutions per minute of orbital shaker (rpm)
20%	2	10	70
40%	4	10	
50%	5	10	
20%	2	10	100
40%	4	10	
50%	5	10	

2.4. Immobilization of *B.sphaericus* with Palm Oil Fuel Ash

About 40% of POFA (w/v) was mixed with 10^9 cell/ml bacterial suspension and incubated for 1 hour in an orbital shaker (100rpm) to allow the attachment of bacteria on POFA completed. The mixture then will be centrifuged and saline solution will be discarded. The immobilized POFA with bacteria was then stored in fridge at 4°C for future use.

2.5. Preparation of Mortar Specimens

Preparation of mortar mixture comprising Ordinary Portland Cement (OPC), sand ($< 2.36 \mu\text{m}$ and retained $1.50 \mu\text{m}$) and deionized water. The ratio of water to cement was 0.5 and cement to sand was 1/3. The mortar specimens were prepared for 50mm x 50mm x 50mm each. The standard mortar mixture was considered as control sample in this study. The mortar specimen containing immobilized *B.sphaericus* with POFA was prepared by substituting OPC in concrete mix by 10%, 20%, and 30% by weight with immobilized POFA mixture. The same proportions were used to prepare the mortar specimen with only POFA. The slump test was done for all pre-mixed before moulding. After 24 hours, all the specimens were de-molded and then cured in urea yeast extract medium for 7 days in open air room. The proportions of mortar mixes are tabulated in table 2.

Table 2. Mix proportion of mortar mixes.

Mixes	Mix proportion (g)				
	Cement	Sand	Water	POFA	POFA+BS
Control	500	1500	250	0	0
10% POFA	450	1500	250	50	0
20% POFA	400	1500	250	100	0
30% POFA	350	1500	250	150	0
10% POFA+BS	450	1500	250	0	50
20% POFA+BS	400	1500	250	0	100
30% POFA+BS	350	1500	250	0	150

2.6. Result Analysis

2.6.1. Compressive Strength. The compressive strength of all specimens was tested after 3 and 7 days of curing. The compressive strength test was conducted according to ASTM C109 (ASTM, 2013).

2.6.2. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) Spectroscopy. Scanning electron microscope (SEM) was performed to observe the microstructure of samples. Moreover, analysis of quantitative elemental composition was performed using energy dispersive x-ray spectroscopy (EDX), which was equipped with SEM instrument. Before performing SEM, the crushed mortar samples were polished and cleaned by ultrasonic cleaner to prevent noise during scanning. To prevent image disturbances, specimens were coated with a thin layer of gold using sputter coater and then the samples were placed into the chamber.

3. Result and Discussion

3.1. Characterization of Palm Oil Fuel Ash

3.1.1. Brunauer-Emmet-Teller (BET) Surface Area Analysis. The BET specific surface area, average pore diameter and pore volume of palm oil fuel ash were found to be 4.909 m²/g, 0.16874 μm and 0.029471 cm³/g, respectively. While the width and length of *B.sphaericus* were in the range of 0.1-1.0 μm and 1.0-5.0, respectively. Thus, the bacteria could be immobilized inside the pore of the POFA.

3.2. Optimization of Immobilization Methodology

Bacteria can be embedded directly into mortar mix during preparation and casting but its microbial metabolic activity will be affected due to high pH of mortar environment and dry condition of mortar become vulnerable to bacteria [36]. Adding bacteria directly to the mortar mix in suspension will limit their life-time due to cement hydration leading to cement sand matrix pore diameter reduction and insufficient nutrients to precipitate calcite crystals. Therefore, it is important to have a protective vehicle for *B.sphaericus* before adding it to cement mortar mixture by immobilizing the *B.sphaericus* with POFA which will prolong their life-time. Thus, the immobilization process was optimized to obtain the optimum parameter.

3.2.1. Percentage of Immobilization. The percentages of immobilization at different POFA concentration and different agitation rate for 100 minutes are calculated and recorded as shown in figure 3. Different POFA concentration and different agitation rate (rpm) for 100 minutes were considered to study the optimum parameter for immobilization methodology. From figure 3, the result showed that the optimum POFA concentration was 40% compared to others. POFA concentration by 20% was most likely not providing enough surface area for immobilizing the *B.sphaericus*, while 50% of POFA concentration was too high. The mixture of immobilization became too thick and it decreased the cell-POFA interaction thus resulting in lower immobilization percentage. For agitation rate of 70 rpm and 100 rpm, the percentage of cells attached on POFA increased with an increase in agitation rate. At 100 rpm of agitation rate, *B.sphaericus* was able to interact with POFA to form physical adsorption due to electrostatic forces or covalent binding between its cell membrane and POFA sufficiently compared to that of 70 rpm. Low agitation rate slowed down the mixing process and cells have limited interaction with the solid carrier. From this study, it can be seen that the highest percentage of immobilization was obtained at 40% of POFA concentration in bacterial suspension with 100 rpm agitation rate at 60 minutes of 98.53% ±1.4. This parameter was used in further study.

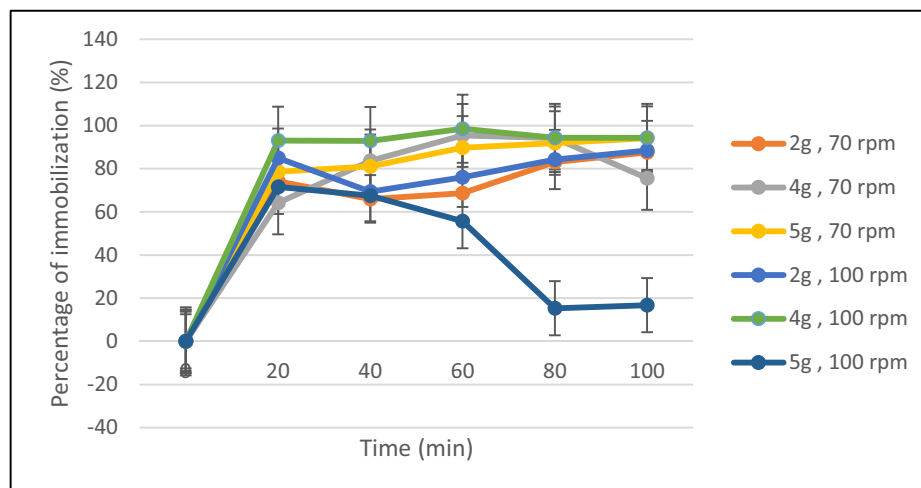


Figure 3. The graph of percentage of immobilization (%) at different POFA concentration and agitation rate for 100 minutes.

3.2.2. Fourier Transform Infrared (FTIR) Analysis. The attachment of *B.sphaericus* with POFA was then analyzed using Fourier Transform Infrared (FTIR) analysis. The FTIR spectra of *B.sphaericus*, POFA, immobilized *B.sphaericus* with POFA and washed immobilized *B.sphaericus* with POFA were compared to verify the immobilization process as shown in figure 4.

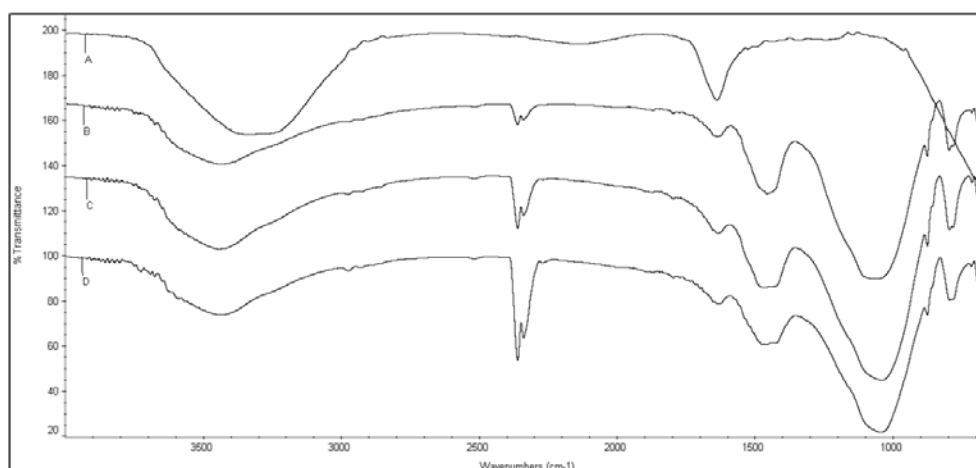


Figure 4. FTIR spectra of a) *Bacillus sphaericus*, b) palm oil fuel ash, c) immobilized *B.sphaericus* with POFA, and d) washed immobilized *B.sphaericus* with POFA.

The spectrum of each sample was combined for comparison as depicted in figure 4. The spectrum of both *B.sphaericus* and POFA were located between $3000\text{--}3500\text{cm}^{-1}$ and $1600\text{--}1700\text{cm}^{-1}$, respectively, which indicated hydroxyl group (3400cm^{-1}) and carboxyl group (1700cm^{-1}). In comparison with the peaks of raw POFA, the peak for immobilized *B.sphaericus* with POFA was slightly broader. This could be due to the bacteria attached on the POFA pore or surface resulting in increased of both functional

groups. However, both peaks returned to their original state when the sample was washed for several times as shown in Figure 4(d) as the bacteria detached from the POFA pore or surface.

3.2.3. Viability of Immobilized *B.Sphaericus* with Palm Oil Fuel Ash. Before adding the immobilized *B.sphaericus* with POFA into cement mortar mixture, it is important to determine the viability of the *B.sphaericus* after it was immobilized and dried in the desiccator. Figure 5 shows the presence of *B.sphaericus* colonies when cultivated after immobilization. This proved that POFA provided a protective microenvironment to the *B.sphaericus* and allow it to have longer life-time due to its high specific surface, porous and spongy surface [8].



Figure 5. Cultivation of immobilized *B.sphaericus* with palm oil fuel ash

3.3. Self-healing mortar

3.3.1. Flow Table Test. After mixing process, the workability of mortar mixture was determined by flow table test and the final diameter of mixture flow were recorded as in table 3. Workability refers to the ability of the mortar mixture to be spread.

Table 3. Table of diameter of sample mixture after flow table test.

Sample	Diameter after test (mm)
CONTROL	133
10% POFA	145
20% POFA	140
30% POFA	130
10% POFA+BS	155
20% POFA+BS	160
30% POFA+BS	165

*POFA+BS = immobilized *B.sphaericus* with POFA

Based on table 3, the workability of mortar with 30% POFA+BS of 165 mm was the highest compared to control mortar and others which could be due to the finer size of POFA used in mortar compared to cement. Water demand will increase with an increase in POFA replacement level due to the augmentation of surface area of fly ash particles [38]. Besides, the workability was improved by additional moisture content from the immobilized *B.sphaericus* with POFA. A sufficient workability for mortar is

around 165-170 mm. If the flow exceeds these limits, that particular pre-mixture has very low workability, high in fluid content and not suitable for construction use [39]. Thus, the results demonstrated that all the samples showed good workability.

3.3.2. Compressive Strength. After 3 and 7 days of curing, all the mortar specimens were subjected to compressive strength test. The result obtained was recorded as displayed in figure 6.

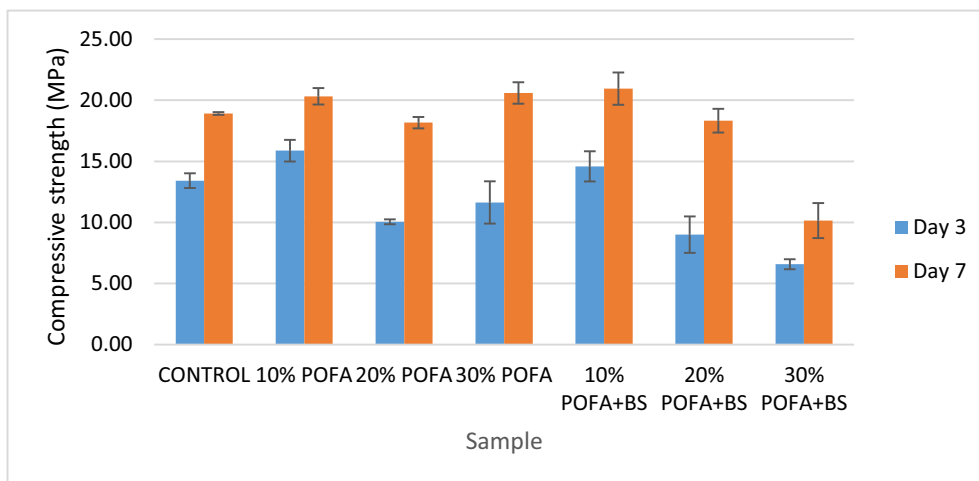


Figure 6. The graph of compressive strength (MPa) of the different sample at 3 and 7 days.

After 3 days of curing, the compressive strength of 10% POFA increased by 18.26% higher than the compressive strength of control mortar which was 15.87 ± 0.88 MPa which was attributed to pozzolanic reaction. High amount of SiO_2 content in POFA reacted with calcium hydroxide to produce more calcium silicate hydrate (C-S-H) gel than that of control and other samples [40]. Then, after 7 days of curing, the compressive strength of 30% POFA was 20.59 ± 0.88 MPa, higher than other mortars incorporating POFA. Generally, replacement of 10% to 30% of OPC by POFA resulting in higher compressive strength compared to the control sample. High POFA content in mortar leads to high production of secondary C-S-H gel. Due to high binding capacity of C-S-H gel, it is able to strongly hold the cement and aggregates together producing high strength mortar [12]. However, if the POFA content exceeds 30%, the mortar produced had low strength due to less cement content and thus affected the hydration process in samples. As a result, less C-S-H gel was produced [1].

Based on figure 6, the compressive strength of 10% POFA+BS at day 3 was 8.72% higher than that of control mortar which was 14.59 ± 1.23 MPa. The sample strength was contributed to pozzolanic reaction and biomineralization of *B.sphaericus*. However, it is still considered as low strength since the strength was lower than mortar with POFA sample which most likely caused by lag phase of the *B.sphaericus* growth. Lag phase is the adaptation period of the *B.sphaericus* to a new environment after it was embedded into mortar mixture. At this level, the number of cell changes and population growth is very low or approaching to zero [41]. However, the cells are not inactive but it has low cell activity. Thus, the lower the cell activity, the lower the mineral crystals or CaCO_3 will be precipitated resulting in lower compressive strength. Then, after the sample was cured for 7 days, the compressive strength of 10% POFA+BS remained the highest which was 20.95 ± 1.32 MPa. In fact, it was higher than compressive strength of 30% POFA sample. This may be due to continuous biomineralization process. Previously, the samples that have been cured only for 3 days have lower biomineralization process because of the lag

phase. However, after 7 days of curing, the immobilized *B.sphaericus* then was moved to the next phase which is log phase. In this phase, the population growth and cell activity were at optimum level. Thus, biomineralization will be higher forming more CaCO_3 to fill in micro pores and cracks [42].

3.3.3. Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy. Scanning Electron Microscopy (SEM) was carried out to determine the microstructure effect on different mixture proportions of mortar samples. Meanwhile, the elements present on sample surface were determined using Energy Dispersive X-ray (EDX) Spectroscopy analysis. Only the sample that have highest compressive strength at each age were subjected to this analysis such as CONTROL at day 3, 10% POFA at day 3, 10% POFA+BS at day 3, CONTROL at day7, 30% POFA at day 7, 10% POFA+BS at day 7 as shown in figures 7 and 8.

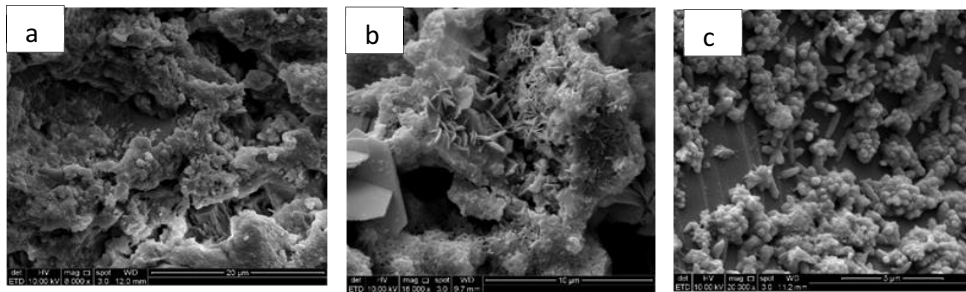


Figure 7. SEM micrograph of: a) control sample at day 3, b) 10% POFA at day 3, c) 10% POFA+BS at day 3.

Figure 7(a) shows the SEM micrograph of control sample at day 3. The magnification was set at 8000 times. Then, the microstructure of 10% POFA at day 3 was obtained as shown in Figure 7(b). The SEM micrograph was taken at 16000 times magnification. From the micrograph, there were flocs-like C-S-H gels formed in the matrix of mortar. These C-S-H gels were well connected to each other to filling the pores of the mortar [33]. The observation for 10% POFA+BS was recorded as in figure 7(c). The SEM micrograph was taken at 20000 times magnification. Based on the micrograph, there were whitish precipitations clumping around the bacteria cells on the surface of the sample. This indicated the precipitation of CaCO_3 by the immobilized *B.sphaericus* on the surface of the POFA.

Table 4. Atomic percentage (%) of different sample at day 3.

Element	Atomic (%)		
	Control (3)	POFA (3)	POFA+BS (3)
C	18.36	11.58	12.14
Mg	0.14	0.33	0.28
Al	1.24	1.19	4.17
Si	11.06	15.16	11.7
K	0.93	1.41	2.56
Ca	2.27	4.79	4.99
O	64.99	63.68	62.64

All those SEM micrograph shown in figure 7 was supported with EDX analysis. Table 4 is the summary of atomic percentage of major elements analyzed by EDX analysis for all samples at day 3. Atomic percentage is the number of atoms of that element compared by the total number of atoms in the

sample within the micrograph frame or magnification. To perform EDX analysis, all the micrographs of samples were taken at 200 μ m size. It can be seen from the table, atomic percentage of silica (Si) was the highest in POFA sample which was 15.16% compared to others. This indicated high amount of C-S-H gels formed. While, atomic percentage of calcium (Ca) was the highest in POFA+BS sample which was 4.99% compared to others due to the precipitation of CaCO₃.

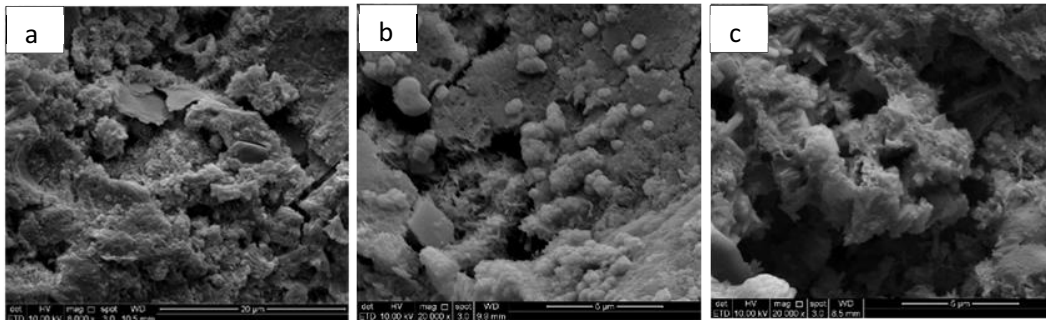


Figure 8. SEM micrograph of; a) control sample at day 7, b)30% POFA at day 7, c) 10% POFA+BS at day 7.

Figure 8(a) depicts the SEM micrograph of control sample at day 3. The magnification was set at 8000 times. Then, the microstructure of 30% POFA at day 7 was obtained as shown in Figure 8(b). The SEM micrograph was taken at 20000 times magnification. From the micrograph, again there were flocc-like C-S-H gels formed in the matrix of mortar same like samples containing POFA at day 3. However, the C-S-H gel formed seem denser and more than sample before. Besides, they were connected to each other very well. Thus, the binding effect might become stronger. This may be due to high POFA content in sample which provided high pozzolanic reaction. Next, the observation for 10% POFA+BS was recorded as shown in Figure 8(c). The SEM micrograph was taken at 20000 times magnification. Based on the micrograph, again there were whitish precipitations clumping around the bacteria cells on the surface of the sample just same with the previous sample. This indicated the precipitation of CaCO₃ by the immobilized *B.sphaericus* on the surface of the POFA. It can be seen in the micrograph that the precipitated CaCO₃ covered up the micro crack of the sample. When the empty space occupied with CaCO₃, it enhanced the strength of the mortar specimens.

Table 5. Atomic percentage (%) of different sample at day 7.

Element	Atomic (%)		
	Control (7)	POFA (7)	POFA+BS (7)
C	13.87	14.15	14.51
Mg	0.34	0.26	0.3
Al	1.15	0.83	8.16
Si	12.44	14.15	7.11
K	0.61	0.76	1.18
Ca	3.83	4.05	6.22
O	63.62	64.44	62.96

All those SEM micrographs shown in figure 9 were supported with EDX analysis. Table 5 summarizes the atomic percentage of major elements as reported by EDX analysis for all samples at day 7. Atomic percentage refers to the number of atoms of that elements compared by the total number of

atoms in the sample within the micrograph frame or magnification. To perform EDX analysis, all the micrographs of samples were taken at 200 μm size. It can be seen from the table, atomic percentage of silica (Si) was the highest in POFA sample of 14.15% compared to others. This indicated high amount of C-S-H gels formed as the curing period increased. While, atomic percentage of calcium (Ca) was the highest in POFA+BS sample of 6.22% compared to others due to the precipitation of CaCO_3 .

4. Conclusion

In conclusion, the *Bacillus sphaericus* was immobilized with palm oil fuel ash (POFA) and the immobilization parameter was optimized. The optimum POFA concentration (w/v) was 40% in 10^9 Cell/ml bacterial suspension in saline solution (NaCl, 8.5g/l). The immobilization process needs to be done in an orbital shaker with 100 rpm for 60 minutes. The attachment of *B.sphaericus* with POFA was then analyzed by FTIR. Next, the differences between normal mortar, mortar with POFA and mortar with immobilized *B.sphaericus* with POFA were investigated in terms of flow table diameter, compressive strength and atomic percentage. The highest flow table diameter was obtained from the 30% POFA+BS which was 165 mm. Then, the strength of the sample was further studied. The highest compressive strength at day 7 was obtained by 10% POFA+BS of 20.95 ± 1.32 MPa. Then, the morphology of pre-mixture was visualized by SEM. From the SEM micrograph, the formation of C-S-H gel present in mortar samples displayed by some flocs-like materials. While, the SEM micrograph of mortar with immobilized *B.sphaericus* with POFA sample showed the whitish mineral crystals clumping around the bacteria conforming the precipitation of CaCO_3 through biomineralization process. The atomic percentages of sample were then analyzed by EDX analysis. The 10% POFA+BS sample was confirmed to had precipitation of CaCO_3 when calcium (Ca) atomic percentage was the the highest (6.22%). At the same time, the silica (Si) atomic percentage was 7.11% indicating the formation of C-S-H gel resulting in double effect of self-healing in the sample. Last but not least, the mechanism of self-healing between *B.sphaericus* and POFA was studied. The addition of POFA into mortar mix resulting in the formation of C-S-H gel that acted as binding agent in the mortar matrix. While, biomineralization of *B.sphaericus* proved that precipitation of calcium carbonate (CaCO_3) occupied the pores in the mortar.

References

- [1] Muthusamy K, Zamri N, Zubir M A, Kusbiantoro A and Ahmad S W 2015 *Procedia Engineering* **125** 804–810
- [2] Bamaga S O, Hussin M W and Ismail M A 2013 *KSCE J. Civ. Eng.* **17**(7) 1708–1713
- [3] Safiuddin M, Abdus Salam M and Jumaat M Z 2011 *J. Civ. Eng. Manag.* **17**(2) 234–247
- [4] Tangchirapat W, Tangpagasit J, Waew-kum S, Jaturapitakkul C 2003 *KMUTT Res. Dev. J.* **26** 459–473
- [5] Usman J, Rahman A, Sam M and Sumadi S R 2015 *Sustain. Environ. Res.* **25**(1) 47–52
- [6] Ranjbar N, Mehrali M, Behnia A and Alengaram U J 2014 *J. Mater. Design* **59** 532–539
- [7] Rajak M A A, Majid Z A and Ismail M 2015 *Procedia Manufacturing* **2** 512–518
- [8] Thomas B 2017 *Renew. Sust. Energ. Rev.* **80** 550–561
- [9] Altwair N M, Johari M A M and Hashim S F S 2014 *Mater. Struct.* **47** (4) 667–682
- [10] Altwair N M, Johari M A M and Hashim S F S 2012 *Constr. Build. Mater.* **37** 518–525
- [11] Abdullah M W, Hussin F, Zakaria R and Muhamad Z A H 2006 *6th Asia Pacific Structural Engineering and Construction (APSEC 2006)* (September) 5–6
- [12] Goñi and Guerrero A 2007 *J. Am. Ceram. Soc.* **90** (12) 3915–3922
- [13] Chong J Z, Sutan N M and Yakub I 2012 *UNIMAS J. Civ. Eng.* **4**(3) 6–10
- [14] El-bendary M 2017 *J. Basic Microbiol.* **46**(2) 158-170
- [15] Nagar I 2010 *J. Econ. Entomol.* **96**(4) 1039-1044

- [16] Arunachalam K D, Sathyanarayanan K S, Darshan B S and Raja R B 2010 *Int. J. Eng. Sci. Technol.* **2**(3) 270–277
- [17] Jonkers H M 2007 *Self-healing concrete: A Biological Approach* (Netherlands: Springer)
- [18] Jonkers H M and Thijssen A 2010 *2nd International Symposium on Service Life Design for Infrastructure 4-6 October 2010, Delft, The Netherlands* (October) 833–840
- [19] Luhar S and Gourav S 2015 *J. Civ. Eng. Res.* **5**(3) 53–58
- [20] De Muynck W, De Belie N and Verstraete W 2010 *Ecol. Eng.* **36**(2) 118–136
- [21] Bang S S, Galinat J K and Ramakrishnan V 2001 *Enzyme Microb. Tech.* **28**(4-5) 404-409
- [22] Rodriguez-Navarro C, Rodriguez-Gallego M, Ben Chekroun K and Gonzalez-Munoz M T 2003 *Appl Environ Microbiol.* **69** 2182–2193
- [23] Wu M, Johannesson B and Geiker M 2012 *Constr. Build. Mater.* **28**(1) 571-583
- [24] Andalib R 2015 *The Characteristics of Structural Concrete using Bacteria Improvement* PhD Thesis Universiti Teknologi Malaysia
- [25] Talaiekhazan A, Keyvanfar A, Shafaghat A, Andalib R, Majid M Z A and Fulazzaky M A 2014 *JETT* **2**(1) 1–11
- [26] Leenen E J T M, Dos Santos V A P, Grolle K C F, Tramper J and Wijffels R H 1996 *Water Res.* **30**(12) 2985–2996
- [27] Lawrence M and Jiang Y 2017 *Bio-aggregates Based Building Materials* (Switzerland: Springer)
- [28] Wang J Y, Snoeck D, Van Vlierberghe S, Verstraete W and De Belie N 2014 *Constr. Build. Mater.* **68** 110–119
- [29] Seifan M, Samani A K and Berenjian A 2016 *Appl. Microbiol. Biot.* **100**(6) 2591–2602
- [30] Van Tittelboom K, De Belie N, De Muynck W and Verstraete W 2010 *Cem. Concr. Res.* **40**(1) 157–166
- [31] Jiang Z, Li W, Yuan Z and Yang Z 2014 *J. Wuhan Univ. Technol.* **29**(5) 938–944
- [32] Ramachandran S K, Ramakrishnan V and Bang S S 2001 *ACI Mater. J.* **98**(1) 3–9
- [33] Cizer O, Campforts J, van Balen K, Elsen J and Gemert D 2007 *BMC* **8** 589–599
- [34] Hou D, Ma H and Li Z 2015 *Adv. Cem. Res.* **27**(3) 135–146
- [35] Chen J J, Thomas J J and Jennings H M 2015 Preparation of single-phase C–S–H specimens from hydrated tricalcium silicate pastes Available from http://www.civil.northwestern.edu/people/thomas/pdf/Chen_CSHPrep_CCR_sub.pdf
- [36] Wang J Y, De Belie N and Verstraete W 2012 *J. Ind. Microbiol. Biot.* **39**(4) 567–577
- [37] Hameed B H, Ahmad A A and Aziz N 2007 *Chem. Eng. J.* **133**(1–3) 195–203
- [38] Alsubari B, Shafiqh P and Jumaat M Z 2015 *Materials* **8**(5) 2154-2173
- [39] Costigan A and Pavia S 2014 *International Masonry Conference 2014* 1–11.
- [40] Sata V, Jaturapitakkul C and Kiattikomol K 2007 *Constr. Build. Mater.* **21**(7) 1589-1598
- [41] Skarstad K, Steen H B and Boye E 1983 *J. Bacteriol.* **154** 656–662
- [42] Seifan M, Younes G, Ebrahiminezhad A and Berenjian A 2018 *Bioproc. Biosyst. Eng.* **42**(1) 37-46