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Application of *Proteus mirabilis* and *Proteus vulgaris* mixture to design self-healing concrete

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ABSTRACT

This study investigated two indigenous micro-organisms that can be isolated from soil. The isolated micro-organisms could precipitate calcium carbonate. These micro-organisms were applied to design self-healing concretes. Concrete is one of the most important materials which is used to build structures. Strength and durability of concrete is very important. Hence, a lot of research in this field is being conducted. Although a few reports can be found on the use of different micro-organism to design self-healing concretes, no research has been carried out to isolate suitable indigenous micro-organisms in Malaysia. In this study two strains of microorganisms were isolated from soil. Broken concrete was treated by a medium culture (MC) containing micro-organisms. Results of this study showed that, cracked concrete could be filled by calcium carbonate after treating by a MC containing micro-organisms. However, this treatment is not very effective on the strength of concrete. Results of this study can be used to have a better grasp of biological self-healing concrete, it is extremely important to have cheap and durable materials to build concrete structures in future.

Keywords: Self-healing concrete; *Proteus mirabilis*; *Proteus vulgaris*

1. Introduction

The worldwide use of Portland cement is on the increase. On the other hand, cement factories cause global warming by the emission of CO_2 into the atmosphere. Hence, there is a necessity to use new materials to repair reinforced concrete structures. Self-healing is defined as the ability of a material or surface to repair damages automatically or autonomously [1]. Self-healing has become an object of active investigation in recent years [1–8]. Self-healing behaviors are divided into two categories, natural and manmade. Manmade self-healing materials are further divided into two other categories, chemical and biological self-healing materials. Self-healing materials are created using polymers [9–11], ceramics [12],

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metals [13,14], and their composites, as well as coatings [7,15,16]. Self-healing mechanisms utilized in the design of these materials draw many concepts from the related field of crack healing [4]. Nowadays, several methods have been developed to make self-healing materials. For example, the use of capsules containing healing agents or using liquid circulation of healing agent in vessels throughout self-healing material. Cracks can rupture the capsules or vessels, and release the healing agents that glue the crack opening. In the biological self-healing mechanisms instead of a self-healing agent, spores of micro-organisms are applied. Cracks can rupture the capsules or vessels containing microorganism spores. The released spores faced with water and nutrients, start growing in cracks. Microorganisms can release some enzymes to convert calcium to calcium carbonate. Calcium carbonate can pack and glue the cracks.

The micro-organisms that are suitable to use in self-healing concretes have to have a strong ability to produce calcium bicarbonate. Calcium carbonate crystal formation takes place within or outside the micro-organism cells. Although some animal or plant cells can also form crystals inside or outside themselves, they are not suitable for use in biological self-healing systems, since they require complex conditions for growing when compared to micro-organisms. Almost all protozoa and some groups of algae and bacteria are able to produce biological calcium carbonate crystals [17–20].

Some bacteria are capable of performing metabolic activities which thereby promote precipitation of calcium carbonate in the form of calcite. Calcite is a carbonate mineral and the most stable polymorph of calcium carbonate ($CaCO_3$). Bacteria can grow in various conditions and they are the most suitable strain to be used in biological self-healing concrete. According to Chahal et al. [21], it was predicted that bacterial calcium carbonate precipitation occurs as a by-product of common metabolic processes such as urea hydrolysis. Bacterial deposition of a layer of calcite on the surface of the specimens results in a decrease of capillary water uptake and permeability towards gas. The type of bacterial culture and medium composition has a great impact on calcium carbonate crystal morphology. Microbial mineral precipitation (biodeposition) involves various micro-organisms, pathways, and environments. Considerable research on carbonate precipitation by bacteria has been done with ureolytic bacteria. These bacteria are able to influence the precipitation of calcium carbonate by the production of urease enzyme. This enzyme catalyzes the hydrolysis of urea to CO_2 and ammonia, resulting in an increase of pH and carbonate concentration in the bacterial environment [22]. Immobilization technique for remediation of cracks in concrete, where microbial cells are encapsulated in polymers, has been adapted to close the gap to enhance the strength for selective concentration [23]. Microbial calcite precipitation occurs as a by-product of common microbial metabolic process, such as urea hydrolysis, photosynthesis, and sulfate reduction. These different metabolic processes increase the alkalinity (pH and dissolved inorganic carbon), and thereby favoring the calcium carbonate precipitation [24]. Calcium carbonate precipitation is a general process in the bacterial world under appropriate conditions [23]. Several bacteria and fungi can induce precipitation of calcium carbonate extracellularly through a number of processes that include photosynthesis, ammonification, denitrification, sulfate reduction, and anaerobic sulfide oxidation [25,26]. *Bacillus pasteurii* produces intracellular urease which constitutes nearly 1% of the cell dry weight [27]. *B. pasteurii*, a common soil bacterium can induce the precipitation of calcite. As a microbial sealant, $CaCO_3$ exhibited its positive potential in selectively consolidating simulated fractures and surface fissures in granites and in the consolidation of sand. Biodeposition of a calcium carbonate layer on degraded lime stone by five different strains of the *Bacillus sphaericus* group and one strain of *Bacillus lentus* was studied [28]. It was found that *Bacillus* strains were capable of depositing calcium carbonate, but in different amounts. Weathered concrete samples, made with Portland cement or with blast-furnace slag cement and fouled by lichens, were treated with *Thiobacillus* bacteria and an appropriate nutrient (nitrogen and carbon) by submersion or sprinkling. Biomineralization of calcium carbonate is one of the strategies to remediate cracks in building materials because cracks not only influence the service durability of the concrete structure, but are also harmful to the structural safety [29]. Bacterial deposition of a layer of calcite on the surface of the specimens resulted in a decrease of capillary water uptake and gas permeability [30]. The surface deposition of calcium carbonate crystals decreased the water absorption with 85% depending on the porosity of the specimens [30].

Although few studies have been conducted on biological self-healing concretes, isolation of indigenous micro-organisms and addition of micro-organisms directly into fresh concrete are yet to be studied in Malaysia. The main aim of this study is to evaluate the self-healing ability of concrete using isolated micro-organisms from Malaysian soil. During this study, three micro-organisms were isolated from the soil. These micro-organisms were added to concrete specimens in two different ways. (1) Concrete was

made by a common method (without bacteria) and subsequently, concrete specimens were treated with-MC containing micro-organisms. (2) Microorganisms were directly mixed with fresh concrete. Then, the results were compared with each other.

2. Materials and methods

The isolated micro-organisms were inoculated into MC with mixed culture. Then MC containing micro-organisms was incubated in a shaker incubator with 160 rpm for 24 h. The MC containing micro-organisms was applied to prepare the concrete specimens. By adding agar-agar to the above-mentioned formulation, it was solidified. A small amount of isolated micro-organisms was inoculated onto plates containing solidified MC. Then, the plates were incubated for 48 h. The micro-organisms were observed through microscope up till calcium carbonate precipitation.

2.1. Micro-organisms isolation and purification

In this study, a special MC containing 20 g/l urea, 2.12 g/l calcium carbonate, 10 g/l ammonium chloride, 25 g/l calcium chloride, and 3 g/l nutrients broth was prepared. MC was autoclaved to disinfect. By adding agar-agar to MC formulation, it was solidified. Both liquid and solidified forms of MC were used in this study.

Four samples were taken from 5-cm-deep soil in the Universiti Teknologi Malaysia (UTM) campus. All these samples were taken from the surrounding area of UTM River. Samples were transferred to the laboratory in a cool box (4˚C). All samples were separately suspended into sterile physiological serum (9 g NaCl in one liter of water) and were completely mixed by a shaker with 160 rpm for 30 min. After that one ml of each sample was transferred into 250-ml Erlenmeyer flasks containing 100 ml of MC. The Erlenmeyer flasks were incubated for 48 h by a shaker incubator with 160 rpm at 28˚C. After 48 h, by a laboratory loop, 0.1 ml of each Erlenmeyer was transferred onto plates containing solidified MC. Then, the plates were incubated for 24 h at 28˚C. After 24 h, many colonies of micro-organisms with different morphologies and colors grew on the solidified MC. Liner culture method was applied for the purification of micro-organisms. Using liner culture, colonies with same color and morphology were transferred onto fresh plates containing solidified MC and they were incubated at 28˚C for 24 h. This process was frequently repeated until the colonies in each plate had same color and morphology. Finally, plates containing pure micro-organisms were kept in a refrigerator maintained at a temperature of 4˚C.

All micro-organisms were tested for production of urease enzymes. Only the micro-organisms that were urease positive were selected for identification and further experiments in this study. Identification of micro-organisms was based on a protocol explained in [31].

2.2. Treating concrete specimens by MC

Six 10 × 10 concrete specimens were prepared by standard methods. The strength and density of all concrete specimens were measured. Then, the specimens were loaded almost to the breaking point. Many cracks were propagated into concrete specimens due to this loading. After that, three concrete specimens were treated by MC containing micro-organisms for 30 days. On the other hand, three other concrete specimens were treated by MC without micro-organisms as control specimens. After 30 days, the amount of strength and density in all concrete specimens was measured again.

2.3. Adding MC into fresh concrete specimen

Thirty 10 × 10 concrete specimens were prepared. Instead of water, MC containing micro-organisms was applied to prepare 15 of the concrete specimens. The other 15 were prepared with MC without micro-organisms, as control specimens. These specimens were subsequently tested for strength and concrete density on the 1st, 7th, 14th, 21st and 28th day. Each test was repeated three times and the average results were reported. Further, broken concrete specimens were treated in drinking water for 30 days. Then, the specimens were tested again for strength.

2.4. Analytical methods

Concrete specimen strengths were measured by a standard compressive strength machine concrete (ARD 2000). Density of concrete specimens was also measured by the Pundit Lab which is an ultrasonic pulse velocity (UPV) test instrument. This instrument is used to examine the quality of concrete. Portland cement type 3 was used for all of experiments in this study.

3. Results and discussion

3.1. Evaluation of isolated micro-organisms

During this study, many micro-organisms were isolated from soil. All micro-organisms were tested for

having the ability of urease production. Among the different isolated micro-organisms, only two strains were urease positive. Bacteriological tests confirmed that these micro-organisms belonged to bacteria. Micro-organisms in mix-culture were transferred onto the solidified MC. After 48 h of incubation at 28°C, micro-organisms were evaluated through a microscope (200× zoom). Many huge and similar particles could be seen in the colonies. These micro-organisms could precipitate calcium carbonate around their cells that is why these micro-organisms seemed bigger than usual microorganisms. A series of biochemical reactions take place to form calcium carbonate as shown in Eqs. (1)–(7) [32].

Urea is hydrolyzed to carbamate and ammonia in the presence of urease as shown in Eq. (1).

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{Urease enzyme}} NH_2COOH + NH_3 \quad (1)$$

Carbamate is spontaneously hydrolyzed to form ammonia and carbonic acid in Eq. (2).

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \quad (2)$$

Carbonic acid is spontaneously hydrolyzed to form carbonate ion and hydrogen ion as shown in Eq. (3).

$$H_2CO_3 + H_2O \leftrightarrow HCO_3^- + H^+ \quad (3)$$

Ammonia spontaneously hydrolyzes to form ammonium and hydroxide ions as shown in Eq. (4).

$$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^- \quad (4)$$

The reaction in Eq. (4) continuously produces hydroxide ion, and this gives rise to pH increase which shifts the overall equilibrium of bicarbonate ion (HCO_3^-) towards the formation of carbonate ions as shown in Eq. (5).

$$HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \leftrightarrow CO_3^{2-} + 2NH_4^+ + 2H_2O \quad (5)$$

Bacteria cell wall has a negative charge and for this reason the cell wall is able to draw positively charged calcium ions (Ca^{2+}) to deposit on their cell wall surface [Eq. (6)]. The Ca^{2+} ions then react with the CO_3^{2-} ions leading to the precipitation of calcium carbonate ($CaCO_3$) at the cell surface as shown in Eq. (7). This precipitation serves as the nucleation site.

$$Ca^{2+} + Cell \rightarrow Cell - Ca^{2+} \quad (6)$$

$$Cell - Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 - Cell \downarrow \quad (7)$$

Micro-organisms in this study were identified according to the standard procedure. Details of micro-organisms identification is shown in Table 1. As can be seen in this table, two strains of bacteria in the name of *Proteus mirabilis* and *Proteus vulgaris* were identified.

P. mirabilis is an important bacterium in medical sciences due to its ability to produce high levels of urease enzyme. Based on Eqs. (1)–(7), urease hydrolyzes urea to ammonia (NH_3) and thus makes the urine more alkaline. If this bacteria affect human body and if it is left untreated, the increased alkalinity can lead to the formation of crystals of struvite, calcium carbonate, and/or apatite in kidney.

P. vulgaris is a bacterium that inhabits the intestinal tracts of humans and animals. It can be found in soil, water, and fecal matter. It is grouped with the enterobacteriaceae and is an opportunistic pathogen of humans. It is known to cause urinary tract infections and wound infections. Therefore, the use of these strains in industrial application to design self-healing concretes needs more studies to avoid the risk of diseases.

3.2. Healing broken concrete by treating with MC

Results show that, cracks were filled by a white residue (Fig. 1(a) and (b)). Many bubbles were released when HCl was poured onto white residue, the reaction between hydrochloric acid and calcium carbonate released carbon dioxide (Eq. (8)). This reaction proved that the white residue is calcium carbonate.

Table 1
Results of micro-organisms identification

Tests	Micr-oorganisms characteristics	
	P. mirabilis	*P. vulgaris*
Urease test	+	+
Gram test	−	−
Morphology test	*Bacilli*	*Bacilli*
Oxidase test	−	−
Lactose fermentation test	−	−
Indole test	−	+
H_2S test	+	+

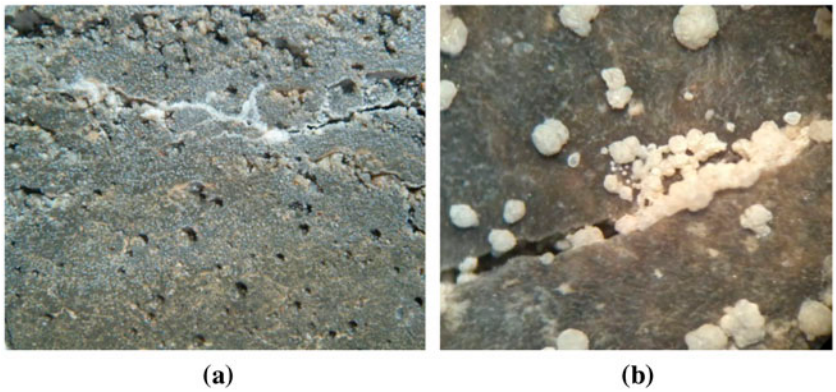

Fig. 1. (a) Filled crack with white residue without zoom, (b) Filled crack with white residue with 40× zoom.

$$CaCO_3 + 2HCl \rightarrow CaCl_2 + CO_2 + H_2O \quad (8)$$

Based on microscopic observations almost all micro cracks were completely filled. The observation showed that although the macro cracks were partially filled, the entrances of surface cracks were completely filled. This can prevent water and chemicals from entering into deeper parts of concrete.

Filling of cracks in the deeper parts of concrete was evaluated by UPV. UPV can be used to identify non-homgeneous conditions in the concrete such as honeycombs, voids, cracks, and frozen concrete. [33]. Therefore, the results of UPV can be used as an indicator of cracks in concrete. The results of UPV before loading, after loading, and after treatment by MC containing micro-organisms are shown in Table 2. As can be seen in this table, the amount of UPV changed from 3,765 to 265 m/s when concrete specimens were loaded to the point of almost breaking. Reduction of UPV shows that many cracks were propagated throughout the concrete specimen. After the treatment of broken concrete specimen UPV was increased up to 3,473 m/s. There was an unexpected result due to filling of cracks by calcium carbonate. These results show that micro-organisms were successful in filling the concrete specimen cracks with the precipitation of calcium carbonate. The micro-organisms could penetrate deep into the concrete and fill the cracks. Hence, 85% improvement in UPV can be observed.

Table 2
Results of UPV in concrete specimens

Type of test	UPV (in m/s)
Before loading	3,756
After loading	265
After treatment by MC containing microorganisms	3,473
Percentage of recovery	85

During the treatment of the concrete specimen, the amount of UPV was measured. The results of this measurement can be seen in Fig. 2. Initially the increasing of UPV rate for both concrete specimens treated by micro-organisms and without micro-organisms was the same. After five days, due to bacterial activities in the concrete specimen treated by micro-organisms, the increase in UPV rate was much higher than the control concrete specimen.

Although, the concrete specimen treated by MC both with and without micro-organisms increased their UPV, the concrete specimens treated by MC with micro-organisms had a higher UPV improvement. There are a number of ways and methods to improve the UPV of concrete specimens without micro-organisms. (1) Filling concrete specimen cracks with the formation of calcium carbonate or calcium hydroxide. (2) Filling concrete specimen cracks with impurities in the

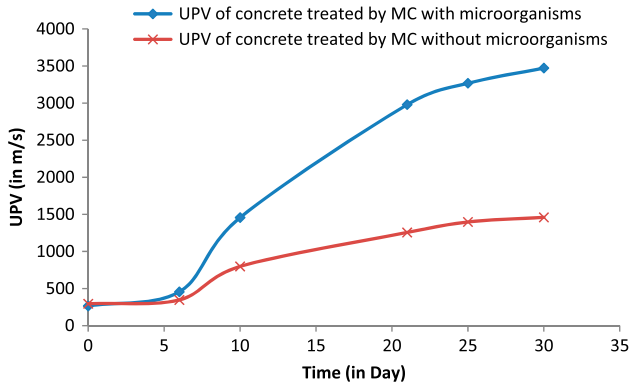

Fig. 2. Amount of UPV during treatment with MC containing micro-organisms and without micro-organisms.

presence of MC such as $CaCl_2$. (3) Filling concrete specimen cracks with hydrated of the unreacted cement or cementitious materials. (4) Filling concrete specimen with the expansion of the hydrated cementitious matrix in the crack flanks (swelling of calcium silicate hydrate gel) [32]. All the above mentioned ways to improve UPV are categorized into natural self-healing processes. As can be seen in Fig. 2, natural self-healing processes are not very effective in improving concrete UPV when compared to using micro-organisms. Isolated micro-organisms in this study could accelerate the improvement of concrete specimen cracks (based on UPV) three times more than the treated concrete specimen without micro-organisms.

In other experiments, concrete strength was determined. Results showed that, treating concrete specimen by MC containing microorganisms did not have a very positive effect on the strength of concrete. Six concrete specimens were loaded to a breaking point and their average strength was equal to 42 Mp. Three concrete specimens were treated with MC containing micro-organisms and other three concrete specimens were treated by MC without micro-organisms (sterile MC). Results elaborated that, the concrete specimens treated with MC containing micro-organisms had an average concrete strength equal to 33 Mp and the concrete specimens treated with MC without micro-organisms had an average strength equal to 29 Mp. It showed only 4 Mp or 10% improvement by filling crackswith micro-organisms.

According to Homma et al. [34], a self-healing concrete specimen containing micro-capsules was loaded to the point of almost breaking. The results of Homma et al. [34] elaborated that self-healing concrete specimen could recover 26% of its original strength. Although the results of this study show a very high percentage of UPV improvement, there was no significant improvement in the strength of concrete when broken concrete specimens were treated with MC containing micro-organisms.

3.3. Designing self-healing concrete by adding MC into fresh concrete

In this part of the study, micro-organisms were directly added to the fresh concrete. The strength and UPV were measured. Results of this part of study are provided in Fig. 3. As can be seen in this figure, significant difference between the strengths of concrete specimens with and without micro-organisms cannot be observed. Using micro-organisms by this method to design self-healing concretes was found to be unsuitable.

No significant difference could be found between UPV of concrete specimens containing MC and micro-organisms and concrete specimens containing MC but without micro-organisms (Fig. 4). As can be seen in Fig. 4, after 30 days of treatment of concrete specimens in water, UVP for both concrete specimens containing MC and micro-organisms and control specimen increased upto 1,400 m/s.

There are many reasons for the failure of this part of study. (1) The isolated micro-organisms did not have enough access to urea and nutrients when they were directly added to concrete. Concentration of MC after mixing with concrete was not suitable for maximum activity of *P. mirabilis* and *P. vulgaris*. (2) Suboptimum pH of concrete was another reason for the failure of this part of study. Based on literature, the best environmental pH of *P. mirabilis* and *P. vulgaris* to grow is around 7.5. But the pH of fresh concrete is between 10 and 13. Therefore, bacteria in this condition could not have maximum activity. To solve this problem, some researchers try to use bacteria spores instead of bacteria [31]. Other researchers suggested the use

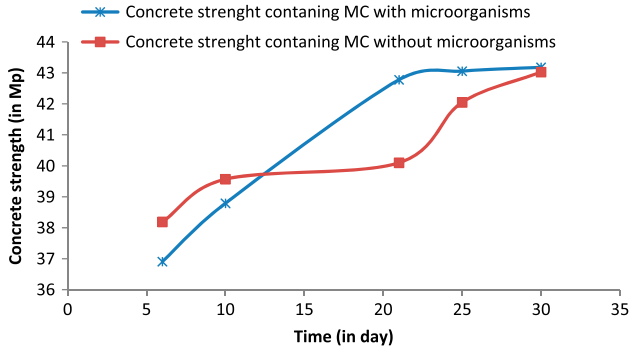

Fig. 3. Concrete containing micro-organisms strength during time.

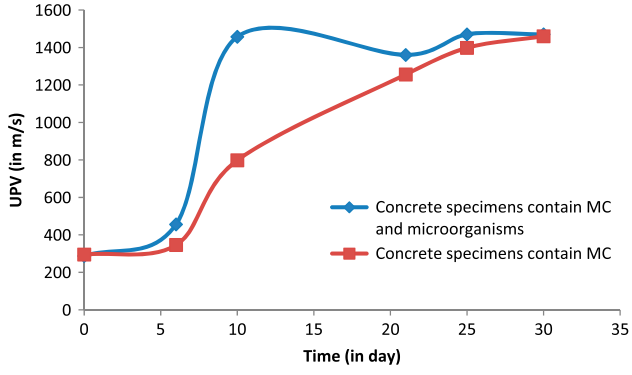

Fig. 4. Amount of UPV during treatment with drinking water.

encapsulated bacteria. However, encapsulation of bacteria is not easy and cheap. (3) Although concrete specimens were floated into water, water cannot flow deeply into concrete. Hence, bacteria had not enough access to water. (4) Based on literature, temperature is increased up to 70˚C during the hardening of concrete specimens. This concrete temperature is mortal to mesophilic bacteria such as *P. mirabilis* and *P. vulgaris* and can extremely increase the decay rate of these bacteria. Above-mentioned reasons can help us to find a suitable method to add isolated bacteria into fresh concrete in the future studies.

4. Conclusions

From the present investigations carried out on two isolated bacteria by the names of *P. mirabilis* and *P. vulgaris*, the following conclusions may be made. These bacteria have the ability to produce urease enzymes which make them completely suitable to calcium carbonate precipitation. Although using these micro-organisms in mixed culture to treat broken concrete showed that they can be very suitable to fill concrete cracks, it was found that they were unsuitable to be added in fresh concrete. Therefore, more studies are needed to complete our knowledge about how these bacteria can be directly used in fresh concrete. These results appear to be a promising technique at this stage of development.

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