Self-Healing Concrete Using Bacteria Calcification from Karst Cave Environment

Ananto Nugroho 1,2 *, Agung Sumarno 1,3, Luna Nurdianti Ngeljaratan 1, Deni Zulfiana 1, Ni Putu Ratna Ayu Krishanti 1, Triastuti 1, Eko Widodo 1

1) Research Center for Biomaterials, Indonesian Institute of Sciences, Jl. Raya Bogor Km. 46, Cibinong, 19611, Indonesia
2) Department of Civil Engineering, Pakuan University, Jl. Pakuan PO Box. 452, Bogor, 16143, Indonesia
3) Department of Civil Engineering, Mercu Buana University, Jl. Raya Kranggan No. 6, Jakarta, 11650, Indonesia

*Corresponding author : ananto@biomaterial.lipi.go.id

Abstract
Karst regions in Indonesia have the uniqueness of the landscape and biodiversity. The karst is formed by the dissolution of rocks and the precipitation of mineral. In the cave, there are ornaments of stalactite and stalagmite which are formed by the process of mineral precipitation. We have isolated, screened, and identified the soil bacterium from the cave environment (Lysinibacillus macroides). These bacteria are able to precipitate calcium carbonate and can be developed as a self-healing agent concrete. We investigated the proportions and the properties of mixtures concrete containing lightweight aggregate and volcanic ash impregnated with bacteria. A comparison study was made by concrete cylinders subjected to compressive strength tests with and without the bacteria. It found that the strength of concrete with bacteria decreased by less than 10.56% for 28 days of cured specimens. This study showed that the effects of bacteria on the strength of concrete are not considerable. However, these bacteria are effective to repair in the microcrack less than 0.3 mm.

© 2019 Indonesian Journal of Applied Chemistry. This is an open access article under the CC BY-NC-SA license (https://creativecommons.org/licenses/by-nc-sa/4.0/).

1. INTRODUCTION
Calcium carbonate is a mineral that represents a large portion of carbon reservoir in the earth. Microorganisms are important active and passive promoters of redox reactions that can influence the precipitation of minerals, including calcium carbonate [1]. Calcium carbonate precipitation has produced from urease activity by microbes. It could be found in the sediments and the cave walls. Some researcher used microbial carbonate precipitation as metal remediation, carbon sequestration, enhanced oil recovery, and construction restoration. [2]. Considerable research on calcite precipitation by bacteria has been investigated by using ureolytic bacteria [3][4]. The ureolytic bacteria hydrolyzed urea into ammonium and carbonate ion. The result of the hydrolysis increased the pH and calcium carbonate precipitation. In nature, biotic urease activity is a widespread phenomenon and includes the actions of bacteria. Urease is an enzyme that hydrolyzes urea and creates calcite in an alkaline environment. Bio-mineralization phenomenon is observed in cave isolates, showing their abilities to precipitate and dissolve calcium carbonate [5].

Bio-calcification has been proposed as an alternative and environmentally-friendly technique to develop self-healing cementitious materials system in recent years [6]. The process of calcite formation was resulted from metabolic activities bacteria which fill up the cracks in the concrete matrix. The research leading to bio-calcification and its ability to self-healing has introduced many methods and applications [7][8]. One of the self-healing methods is impregnation the healing agent that got into porous aggregate with bacterial spores, calcium, and nutrient. These materials were added to concrete [9]. Utilization of these concepts in
concrete leads to the potential invention of a new environment-friendly material called bio-concrete.

Indonesia has the uniqueness of the landscape and biodiversity; one of them is karst cave in Gunung Kidul, Yogyakarta. Soil bacteria are correlated highly with characteristics of the karst ecosystems as represented by their different karst geochemical environments and vegetation [10]. The study of bacterial diversity in the bio-concrete rarely had worked in Indonesia. The purposes of this study are to obtain and to evaluate bacterial isolates for bio-concrete with high urease activity. The present work deals with the comparative study of the compressive strength of concrete subjected to compressive strength tests with and without the bacteria.

2. EXPERIMENTAL SECTION

2.1. Bacterial sources

2.2.1 Isolation and purification

Soil and stalagmites samples were took from Jomblang Cave in Gunung Kidul, Yogyakarta, Indonesia. The medium for the isolation of bacteria was selective medium with the following compositions: 20 g of urea, 3 g of nutrient broth, 10 g of NH₄Cl, 2.12 g of NaHCO₃, 4.41 g of CaCl₂•2H₂O in 1 L distilled water, and 15 g of agar [11]. The culture was incubated at the room temperature (28°C) for three days. The method of streak plate purification was conducted to obtain pure bacterial isolates. Figure 1 below shows the stalagmite where the sample was taken.

![Figure 1. Stalagmite](image-url)

2.2.2 Screening

The screening was carried out by growing the isolates in the urease test medium broth using Hammes method [11]. The media of urease activity could be observed after incubation at 28°C from one to three days. Bacterial isolates that have the urease activity will change the color of the medium from yellow to fuchsia pink. The urease activity was determined by measuring the amount of ammonia released from urea according to the phenol-hypochlorite assay method [12]. The reactions were carried out in test tubes containing 100 µL of the sample, 500 µL of 50 mM urea and 500 µL of 100 mM KH₂PO₄ buffer (pH 8.0) so that the total volume was 1.1 mL. The reaction’s mixture was incubated at a temperature of 37°C for 30 minutes. This reaction was stopped by transferring 50 µL of the action mixture into tubes containing 500 µL solution of phenol-sodium nitroprusside. Alkaline hypochlorite solution 500 µL were added to the tube, then incubated at ambient temperature for 30 minutes. The optical density was measured with a spectrophotometer at λ = 630 nm and compared with a standard curve (NH₄)₂SO₄.

2.2.3 Bacterial Identification

A strain of bacteria used in this research was gram-positive, endospore-forming, and urease positive. The pure cultures of bacteria that have the highest urease activity were used for molecular identification. Identification was carried out using molecular analysis based on 16S rDNA fragments in bacteria. Bacterial DNA isolation was performed using the Polymerase Chain Reaction (PCR) method [13]. Amplification of 16S rDNA fragments was performed using GoTaq (Promega) with primer 27F (5’-AGAGTTTGATCTGTCGGCTCAG-3’) and 1492R (5’-GGTTACCTTGTACGACTT-3’) [14]. PEG precipitation method was used to purify PCR; after that, it continued by cycle sequencing [15]. The result of sequencing cycles was purified using ethanol purification method. Analysis of the nitrogen base sequence used automated DNA sequencer (ABI PRISM 3130
Genetic Analyzer). The Bioedit program processed the data of sequencing result. 16S rDNA sequence homology program searched using Basic Local Alignment Search Tool Nucleotide (BlastN) on the website of the National Center for Biotechnology Information (NCBI).

### 2.2. Materials

#### 2.2.1. Cement

Portland Pozzolana Cement (PPC), which is available in the local market, was used. The cement used has been tested for various properties as per SNI 15-0302-2004.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Volume ratio (PPC: Sand : Split : Hydroton)</th>
<th>W/C</th>
<th>Volcanic ash contain cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>1 : 2 : 3 : 0</td>
<td>0.65</td>
<td>No</td>
</tr>
<tr>
<td>AB</td>
<td>1 : 2 : 3 : 0</td>
<td>0.65</td>
<td>Yes</td>
</tr>
<tr>
<td>BC</td>
<td>1 : 2 : 2.75 : 0.25</td>
<td>0.65</td>
<td>No</td>
</tr>
<tr>
<td>BB</td>
<td>1 : 2 : 2.75 : 0.25</td>
<td>0.65</td>
<td>Yes</td>
</tr>
<tr>
<td>CC</td>
<td>1 : 2 : 2.5 : 0.5</td>
<td>0.65</td>
<td>No</td>
</tr>
<tr>
<td>CB</td>
<td>1 : 2 : 2.5 : 0.5</td>
<td>0.65</td>
<td>Yes</td>
</tr>
<tr>
<td>DC</td>
<td>1 : 2 : 2 : 1</td>
<td>0.65</td>
<td>No</td>
</tr>
<tr>
<td>DB</td>
<td>1 : 2 : 2 : 1</td>
<td>0.65</td>
<td>Yes</td>
</tr>
</tbody>
</table>

#### 2.2.2 Coarse and fine aggregates

All aggregates shall conform to SNI 03-1750-1990. Fine aggregates; used the natural river sand, were washed and screened to eliminate unwanted deleterious material and oversize particle. Natural river sand, according to SNI 03-1968-1990, is zone II with the specific gravity 2.64. This study used the crushed rocks aggregate as coarse aggregate with 20 mm in diameter and 2.65 for the specific gravity.

#### 2.2.3 Lightweight expandend clay aggregates

Hydrotone was used in this study as a replacement for the coarse aggregates. It was a circle which the maximum diameter 10 mm and no pass sieve no 4 (4.75 mm). In the other hand, the specific gravity of Hydrotone is 0.84. The crushed Hydrotone was dried at 115°C for 12 hours, then cooling it to room temperature. Some of these aggregates were subsequently brought under partial vacuum, after which a 150 mM calcium lactate solution was added until all the aggregates were submerged. The aggregates were dried at a temperature of 30°C.

#### 2.2.4 Volcanic ash

The material which was from Mount Kelud eruption in 2014 (particle size 0.053 mm and the specific gravity of 2.26) has been used as a medium for bacterial spore. Volcanic ash was dried overnight at a temperature of about 115°C, followed by cooling to room temperature. An amount of these particles was brought under partial vacuum, which 1 mL of 1.2 x 10⁵ cells mL-1 spores suspension (LMS05) was added per 17 g of ash particles. The particles were dried at a temperature of 30°C. Figure 2 below shows the pore structure in volcanic ash.

#### 2.2.5 Microorganism

Cells cultured and grown in the laboratory for 24 hours. The medium composition for growth of culture was Peptone: 5 g L-1, NaCl: 5 g L-1 and Yeast extract: 3 g L-1.

---

"Self-Healing Concrete using Bacteria Calcification …": Ananto Nugroho, et.al. | 9
code for four different volume ratios. They were code A, B, C and D. In this mix design, hydroton were used as a replacement of coarse aggregate. Code C was concrete without bacteria, and code B were concrete with bacteria. The addition of volcanic ash in concrete was 1.5% by weight of cement. The corresponding identification marks were labeled over the concrete surface. All specimens were water cured for 28 days then tested in the compression machine. The specimens were immersed in water for curing until the compressive strength testing at 28 days. The compressive strength of each specimen was calculated as the mean value of six specimens.

2.4 Microscope observation

A digital microscope and Scanning Electron Microscope (SEM) were used to observe the calcite precipitate on the crack after compressive testing. The crack on concrete was observed during 60 days in the humid environment. This research used digital microscope Dino-Lite Pro AM 413-FIT to measure the size of crack and precipitate of calcite. In addition, SEM JEOL (SM-5310LV) was used to observe the morphology of crystal calcite precipitated.

3. RESULTS AND DISCUSSION

3.1. Microorganism

3.1.1 Urease test

Testing of urease activity used 40 isolates of bacteria. The discoloration of the media was from yellow to bright pink (fuchsia). This color indicated there was a urease activity in the media. 25 of 40 isolates of bacteria result in the bright pink (fuchsia).

3.1.2 Urease activity

The ability to precipitate calcite is directly related to the amount of the enzyme urease produced by the bacteria. The activity of urease happened at 30 minutes after the incubation at 28°C. LMS05 was one of the isolates which were taken from the mud under the stalagmite. It was the highest urease activity than the other isolates. The urease activity of Isolate - LMS05 was 8.94 μgmL⁻¹minute⁻¹. Figure 3 below shows urease activity for each of isolate.

3.1.3 Molecular identification

Phylogenetic analysis positions determined by 16S rRNA sequence of Isolate-LMS5 showed 99% similarity with Lysinibacillus macroides strain CS26, compared to the sequence of DNA in National Center for Biotechnology (NCBI) Gen Bank database, with Max score: 2499; Total score: 2499; Query coverage: 100%; E-value 0.0; Max identities: 1368/1374 (99%); Gaps: 5/1374 (0%). This result confirms that our Isolate-LMS5 corresponds to Lysinibacillus macroides.

Figure 3. Urease activity from 25 of isolate bacteria

3.2. Bio-concrete

3.2.1 Properties of concrete

The compressive strength of concrete tested in compression testing machine at 28 days for control concrete and bio-concrete. The comparison study of compressive strength was made using specimens with and without the bacteria. The test results for concrete properties are shown in Table 2.

The result was not good at the compressive strength. Table 2 shows that the compressive strength decreased at all samples of bio-concrete. The bacteria agent into volcano ash affects slightly reducing the compressive strength. The compressive strength of the concrete with bacteria was 10.56% lower than the control. The decreasing of the compressive strength was caused by bacteria agent, which affected the
character of cement, especially at the initial hardening process of concrete [9].

**Table 2. Properties of concrete specimens**

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Density (kg/m³)</th>
<th>Absorption (%)</th>
<th>Compressive strength (N/mm²)</th>
<th>Loss in strength (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>2199</td>
<td>7.30</td>
<td>15.22</td>
<td>-</td>
</tr>
<tr>
<td>AB</td>
<td>2096</td>
<td>10.09</td>
<td>13.61</td>
<td>10.56</td>
</tr>
<tr>
<td>BC</td>
<td>2096</td>
<td>8.25</td>
<td>15.00</td>
<td>-</td>
</tr>
<tr>
<td>BB</td>
<td>2061</td>
<td>11.85</td>
<td>13.72</td>
<td>8.57</td>
</tr>
<tr>
<td>CC</td>
<td>2007</td>
<td>8.38</td>
<td>12.75</td>
<td>-</td>
</tr>
<tr>
<td>CB</td>
<td>2006</td>
<td>11.20</td>
<td>11.90</td>
<td>6.72</td>
</tr>
<tr>
<td>DC</td>
<td>1961</td>
<td>10.23</td>
<td>11.90</td>
<td>-</td>
</tr>
<tr>
<td>DB</td>
<td>1907</td>
<td>12.36</td>
<td>11.25</td>
<td>5.41</td>
</tr>
</tbody>
</table>

Moreover, the decreasing of the compressive strength could be caused by the concentration of *Lysinibacillus macroides* (1.2 x 10⁵ cell/ml). It was not an ideal concentration to improve compressive strength. The effective of bacteria concentration as crack recovery was not the same with a total of bacteria concentration that is improving the compressive strength. The different type of bacteria, level of bacteria concentration, and mix design of bio-concrete caused a different type of precipitated of calcite in the matrix of concrete [16].

The utilization of a healing agent has not been able to reduce the natural porosity in the concrete. As metabolically active, the bacteria consume the oxygen, although there is not enough available oxygen after curing for 28 days in the pores of the concrete. In general, the pH of fresh concrete is between 11 and 13. The temperature of fresh concrete can go up to 70°C. After drying of concrete, the water content in the concrete will decrease. Therefore, the bacteria should be able to survive in the difficult condition with high pH, temperature, and lack of water. The suitability of the bacteria applied in concrete as a self-healing agent relates to their capacity to form spores [17].

**3.2.2 Microscope observation**

The bacteria will not precipitate calcite in the pores of the concrete as long as they are in normal condition (there is no crack). Calcite precipitation filled in the crack after the concrete was under water for 60 days. Recovery cracks that occur triggered by water and supported by oxygen content. Figure 4 below shows that the crystal precipitation process was able to seal the cracks less than 0.3 mm.

**Figure 4. Crack healing**

**3.2.3 SEM Observation**

The result of SEM shows calcium carbonate precipitated in the crack of concrete. The calcium carbonate precipitated have stages of crystals formation withmorphologies of rhombohedral crystals. The specificity of crystal growth was knowns primarily because of differences in bacterial genera. The crystal growth could be inhibited or altered by the adsorption of proteins, organic matter, or inorganic components to specific crystallographic planes of the growing crystal [18]. Figure 5 below shows the crystal morphology of calcite precipitate.

**Figure 5. Calcite precipitate**
4. CONCLUSION

This research got *Lysinibacillus macrolides*, which have the highest urease activity than the other bacteria. These bacteria were added to concrete by volcano ash. Additions the bacteria on the concrete have affected on the compressive strength and the ability to recover the crack. The compressive strength of bio-concrete was decreased 10.56% than the control samples for 28 days cured specimen. This study showed that the effects of bacteria on the strength of concrete are not considerable. This method is effective to repair micro crack less than 0.3 mm. Moreover, there was needed further research to increase self-healing and compressive strength.

ACKNOWLEDGMENT

We would like thanks to the Jomblang Cave Management, who allowed us to take the sample and the Head of Research Center for Biomaterials, Indonesian Institute of Sciences who support us in this research program.

REFERENCES


