Bacterial from wawolesea hot springs: crack sealing application to concrete

P.E. Susilowati¹, A. Zaeni¹, S. Kartini¹, N.A. Rajiani¹, H. Hermawan¹ and I. N Sudiana²

¹Chemistry Department, Halu Oleo University, Kendari, Indonesia
²Physic Department, Halu Oleo University, Kendari, Indonesia

ahmad.zaeni@gmail.com (corresponding author)

Abstract Concrete is one of the most widely used construction materials for infrastructure, but concrete has the disadvantage of being easy to crack. One of the factors that influence concrete cracks is rain water, because water can enter and seep into the pores. Several studies have been carried out to overcome the cracking of concrete structures; one of them is the addition of bacteria that have calcium carbonate precipitating activity. Calcium carbonate from bacterial metabolism can closed on cracked concrete surfaces. This method is commonly called Microbial Induced Calcite Precipitation (MICP). The source of bacteria in this study is bacteria at Wawalosea hot springs from the limestone mountain. The research is healing of cracks in concrete compare by bacteria MICP and MICP immobilization using alginate-chitin. The results showed that the concrete made with the addition of MICP isolates as much as 1.6x10⁸ cells / mL had a greater compressive strength than the concrete without the addition of isolates. The value of compressive strength and absorbency of concrete water with the addition of MICP isolates has a compressive strength of 25.78 Mpa and addition immobilization MICP has 18 Mpa. So when compared to concrete without the addition of isolates and concrete with the addition of MICP isolates, the value of concrete compressive strength occurred at 100%.

1. Introduction
Concrete is an important and dominant construction material used in building structures. Concrete is a construction material that has many advantages, among others easy to do by mixing cement, aggregate, water and other additives when needed with a certain ratio. Therefore concrete is one of the most dominant construction materials used in building structures. However, most concrete structures are prone to cracking due to environmental conditions. Small cracks on the concrete surface make the structure prone to damage because water can seep through cracks. This can result in reducing the life of concrete structures, thus causing economic losses.

The method commonly used today to repair concrete cracks is the use of synthetic polymers that need to be applied repeatedly, this requires monitoring and expensive costs. Therefore it is necessary to find alternatives to improve the durability of concrete that is more environmentally friendly. Several studies have been carried out to overcome the cracking of concrete structures, one of which is the addition of microbes that have the activity to metabolize calcium carbonate, so that calcium carbonate can freeze on the cracked concrete surface.

Induced Calcite Precipitation Microbial (MICP), is a microbe that can be normalized in concrete. However, when the concrete structure is damaged and water starts to enter through cracks in the concrete, the microbes will multiply due to contact with water and nutrients. This microbe will produce urease which can catalyze the hydrolysis of urea (CO(NH₂)₂) to ammonium (NH₄⁺) and...
carbonate (CO$_3^{2-}$). The cell wall of bacteria is negatively charged, causing bacteria to be able to attract calcium ions (Ca$^{2+}$) from the environment. Calcium ions (Ca$^{2+}$) then react with carbonate (CO$_3^{2-}$) which causes precipitation of calcium carbonate (CaCO$_3$) on the cell surface [1].

**Figure 1. Biological and chemical processes in MICP [2]**

Self-healing concrete is a biological product that will produce lime to repair cracks on the surface of concrete structures. The bacteria commonly used are from the genus *Bacillus*. Self healing agents can form in concrete up to 200 years [3]. However, when the concrete structure is damaged and water begins to seep through the gaps in the concrete, eating spores from bacteria will germinate. Once activated, bacteria begin to eat calcium lactate. An additional advantage is that with the activity of bacteria the amount of oxygen is reduced so that it can increase the durability of steel construction of concrete.

In Southeast Sulawesi, there is a unique hot spring of Wawolesea, which is located on the edge of the beach and mountains of limestone. In hot springs there is a potential to find thermophilic microbes that can live in calcareous conditions. It is possible for microbes to metabolize calcium to form lime deposits. Based on observations, it can be seen on the surface of this hot water that there is limestone deposition. Therefore, it is expected that from Wawolesea hot spring a thermophilic microbe will be able to metabolize calcium carbonate.

The ability of bacteria to live in concrete is an important factor because concrete has a high alkalinity (pH 12-13). The pH of growth genus *Bacillus Acidocaldarius* 8-9 [4]. To maintain the metabolic activity of bacterial cells at high pH, one of them is immobilizing bacterial cells. The immobilization process is carried out by physically placing the bacterial cell in a particular space where the bacterial cell still has catalytic activity.

2. Methods

2.1. Bacteria Growth
Bacteria were inoculated with pour plate (anaerobic) technique on LB (Luria Bertani) media (Pepton 2% (w/v), Yeast Extract 1% (w/v), NaCl 2% (w/v), MgSO$_4$ 1% (w/v), Aquadest, MgSO$_4$.7H$_2$O 1% (w/v) and CaCl$_2$.7H$_2$O 50 g/mL. Bacteria incubated at 47 °C.

2.2. Bacterial Immobilization
Sterile chitin soaked in 0.15 M CaCl$_2$ solution for 3 minutes, remove and drain. Added 20 mL of LB media containing bacterial cells (bacteria mixed with 4% alginate solution). Chitin mixture, cell
culture and alginate, reacted after how much time, filtered. The immobilized matrix was washed in 0.15M CaCl$_2$ solution, and phosphate buffer pH 7, and stored in a phosphate buffer pH 7 at 4 °C [5].

2.3. Urease Test
1 mL of bacterial cells (immobilized or not immobilized) inserted in a test tube. Subsequently, 3 mL of sterilized urea media were added, incubated at 47 °C. The media observe is colour changes at media.

2.4. Estimated Calcium Carbonate Produced
Bacterial cells 50% (immobilized or not immobilized) are inoculated in LB media containing calcite (a mixture of 20 g / L urea and 50 g / L calcium chloride). Furthermore, it was incubated at 47 °C and periodically the amount of calcite formed. The analysis volumetric method, by adding excess acid to the bacterial culture media and then titrated again with a solution of NaOH [6].

2.5. Characterization of Concrete Mixtures

2.5.1. Making concrete with the addition of bacteria
Cement paste and mortar with varied ratio. Bacteria added in various concentrations. Furthermore, concrete is printed. The hardened concrete is then immersed in water at intervals of 0, 14, 28 days.

2.5.2. Water Absorption
Concrete before and after immersion is weighed. Measurement of water absorption (Water Absorption).

2.5.3. Concrete Strength Test
Concrete that has been immerse, then performed a pressure test, using a compression testing machine.

3. Result’s and Discussion
The application of bacterial isolates in a concrete specimen is carried out after it is known that its ability to precipitate calcium carbonate. The results showed that the Wawolesia isolate was able to produce 0.067g / mL of calcium carbonate. These study showed that 1 gram of chitin immobilized bacteria produced 0.0054 grams of calcium carbonate.

Bacterial cell immobilization is a method for confining or physically placing a bacterial cell in a particular space, but the cell still has catalytic activity, so that it can be used continuously or repeatedly. In this study, immobilization of bacterial cells using the entrapment method, i.e. bacterial cells are trapped physically but not chemically bound. This method is based on the entrapment of bacterial cells in a semipermeable polymer matrix so that microorganisms can still carry out their activities[7]. Bacterial cells mixed in alginate solution will be absorbed when alginate form a gel in the presence of divalent or polyvalent cation ions[7]. While chitin used is able to absorb bacterial cells, because chitin has many pores, causing capillary symptoms that can attract the solution and solids that are around it.

Characterization of concrete mixtures and MICP (immobilized and non-immobile bacteria), was carried out to determine concrete mixes that had maximum strength. Concrete quality testing includes compressive strength and water absorption. Good concrete has a low water absorption and large compressive strength. Compressive strength is the most important property for concrete. Compressive strength is the ability of a material to withstand a compressive load.

Water absorption is one of the most important parameters to predict and determine the strength and quality of concrete produced. Good quality concrete has little water absorption where the number of pores on the surface is small and tight.

3
Table 1. Water absorpsi in concrete

| Soaking in water (days) | Water Absorption (%) |           |          |
|-------------------------|----------------------|-----------|----------|
|                         | Concrete control     | Concrete+ MICP (50%) | Concrete + MICP immobilized (50%) |
|                         |                      |           |          |
| 7                       | 13.40                | 4.03      | 6.0      |
| 14                      | 13.55                | 6.25      | 6.0      |
| 28                      | 14.97                | 3.94      | 3.0      |

The results showed that the longer soaking time (28 days) would cause a decrease in water absorption (Table 1), and an increase in the compressive strength of concrete (Table 2). In soaking 7 and 14 days water absorption is still quite high. This condition may be caused by soaking 7 and 14 days, causing concrete cracks, which cause water to enter the concrete pores. Furthermore, MICP bacteria mixed with concrete will use water to metabolize.

Table 2. Compressive strength in concrete

| Soaking in water (days) | Compressive Strength (MPa) |           |          |
|-------------------------|-----------------------------|-----------|----------|
|                         | Concrete control            | Concrete+ MICP (50%) | Concrete+ MICP (50%) |
|                         |                             |           |          |
| 7                       | 8.59                        | 11.72     | 12       |
| 14                      | 10.94                       | 13.28     | 11       |
| 28                      | 11.72                       | 25.78     | 18       |

In theory, good quality concrete has little water absorption because the number of pores on the surface is small and tight. The results obtained are not much different from the research conducted by [8], namely concrete added with *Sporosarcina sphaericus* bacteria which was immobilized using paliureatan has a smaller water absorption compared to control concrete.

Compressive strength is the ability of concrete to accept the compressive force of broad unity. Concrete compressive strength identifies the quality of a structure. The higher the desired level of structural strength, the higher the quality of the concrete produced.

The results showed the strength value of concrete mixed with MICP (immobilized and non-immobilized bacteria) was higher than in the control concrete, although it was better concrete mixed with non-immobilized bacteria. This condition may be caused by immobilized bacteria not spread evenly inside the concrete, so that the calcium carbonate produced is not evenly distributed on the concrete surface.

During the process of bacterial growth, the resulting calcium carbonate will settle to the surface of the bacteria as well as to the cracked concrete pores. After many pores in the concrete are covered in calcium carbonate, the flow of nutrients and oxygen to bacterial cells will decrease, eventually the bacteria in the matrix become dormant. The mechanism of calcium carbonate precipitation begins with the reaction, urea CO(NH$_2$)$_2$ is hydrolyzed to produce NH$_3$ and CO$_2$ (Reaction 1), then spontaneously hydrolyzes NH$_3$ to produce NH$^{+}$ and OH$^-$ (Reaction 2). NH$^{+}$ production will increase pH. Furthermore CO$_2$ will act with OH$^-$ which has been obtained from hydrolysis of NH$_3$ to produce CO$_3^{2-}$ (Reaction 3). In the presence of enough Ca$^{2+}$ and CO$_3^{2-}$ ions, the formation of CaCO$_3$ (Reaction 4) will result. Ca$^{2+}$ ions which are bound by bacterial cell-wall will cause CaCO$_3$ crystals to form in bacterial cells.

\[
\text{CO(NH}_2\text{)}_2 + \text{H}_2\text{O} \rightarrow 2\text{NH}_3 + \text{CO}_2 \\
2\text{NH}_3 + \text{H}_2\text{O} \rightarrow 2\text{NH}_4^+ + 2\text{OH}^- \\
\text{CO}_2 + 2\text{OH}^- \rightarrow \text{CO}_3^{2-} + \text{H}_2\text{O} \\
\text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_3 (s)
\]
This condition is aimed at increasing the compressive strength especially because the consolidation of the matrix in the concrete specimen caused by pores in the concrete has been microbiologically induced by calcium carbonate precipitation. The compressive strength of concrete increases due to the concentration of bacterial cells. This was mainly due to microbes inducing calcium carbonate deposits which gave a two-fold increase compared to controls at 28 days imerse.

References

[1] Amakrishnan V, Ramesh K.P, Bang S S, 2010, Improvement of concrete durability by bacterial mineral precipitation, Proceedings of ICR, 11, Torino, Italy.

[2] De Jong, J T, B M. Mortensen, B C. Martinez and D C. Nelson, 2010, Bio-mediated soil improvement, Ecological Engineering, pp.197-210.

[3] Jonkers, H M, and Schlangen, E, 2009, A Two component Bacteria-Based Self-Healing concrete, Concrete Repair, Rehabilitation and Retrofitting II, pp. 215-220.

[4] Ramakrishnan, V, Panchalan, R K., Bang, S S, and City, R, 2005, Improvement of Concrete Durability By Bacterial Mineral Precipitation, International Conference on Fracture.

[5] Swaisgood, H E and F M L Passos, 1997, Immobilization of Enzymes and Cells, Humana Press. Totowa. New Jersey.

[6] Kumar Jagadeesha B G, R Prabhakara, Puspa, 2013, Bio Mineralisation of Calcium Carbonate by Different Bacterial Strains and Their Application in Concrete Crack Remediation, International Journal of Advances in Engineering and Technology vol. 6: 1, pp 200–213.

[7] Henrikardiyyah, N W, 1996, Kajian Amobilisasi Mikroorganisme Penghasil Protease untuk Solubilisasi Protein hasil Perikanan [skripsi], Bogor : Fakultas Teknologi Pertanian. Ilmu Pangan dan Gizi.

[8] Wang J Y, Tittelboom V V, De Belie and W Verstraetes, 2010, Potential of Applying Bacteria to Heal Cracks in Concrete, International Conference on Sustainable Construction Materials and Technologies.