

# The Utilization of Milk as a Catalyst Material in Enzyme-Mediated Calcite Precipitation (EMCP) for Crack-Healing in Concrete

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**Abstract:** This study modified the Enzyme-Mediated Calcite Precipitation (EMCP) method by adding milk as a catalyst in calcite formation. Cracks in concrete samples were made when the concrete was 28 days with a width of 0.1-0.3 mm. The optimum EMCP solution composed of urease, urea, CaCl<sub>2</sub>, and milk was injected into the cracked concrete sample, and its effect on permeability and compressive strength tests were evaluated. The optimum composition of milk used in the formation of calcite had a milk concentration of 5 gr/l with an initial preparation temperature of 70°C, which produced 26% more calcite mass compared to a basic EMCP solution. After three injection times, it reached 92.23% of the cracked concrete's permeability coefficient value and 98.75% of the planned compressive strength. In conclusion, milk utilization as a catalyst for repairing concrete cracks with the EMCP method can be used.

**Keywords:** Cracking; compressive strength; EMCP; milk; permeability.

## Introduction

Concrete is a material that has strength and durability for construction [1]. However, concrete has a high potential for cracking and thus promotes shrinkage and deflection. High-temperature differences can generate cracking during the drying process. Cracks can promote corrosion in reinforced concrete when the carbonation between chloride ions and CO<sub>2</sub> gas occurs in concrete through existing cracks. The occurrence of corrosion decreases the performance of building structure components [2].

Repairing cracks in concrete, in general, is only done in locations that can be reached by workers [3]. Currently, several methods for crack healing concrete have been developed; one is self-healing concrete. According to Mihashi and Nishiwaki [4], self-healing concrete can be divided into two types, based on the repair mechanism, namely autogenous healing and engineered healing. Autogenous healing is a process that occurs in concrete due to a chemical reaction from the concrete matrix, while engineered healing is obtained by adding specific chemicals and micro-organisms to the concrete mixture, such as the use of bacteria as a healing agent. This method was introduced as the self-healing concrete and a low-cost infrastructure solution, both in construction and maintenance [5].

A self-healing concrete has significant benefits to maintain the sustainability of concrete. It can remediate the crack efficiently, thus reducing cracked concrete's permeability and preventing concrete from corrosion; it also resists temperature fluctuation. The ability of bacteria to convert dissolved organic nutrients into calcite crystals, which later serve to close the cracks, is the main principle of the self-healing method. A previous study has tried to apply a species of *Bacillus* bacteria, namely *Bacillus sp.*, as an independent self-recovery agent with a different bacterial dose. It was reported to close cracks in concrete and maintain the compressive strength [6]. *Bacillus sp.* produces calcium carbonate crystals when cultured on media containing Ca<sup>2+</sup>. In the calcite group *Bacillus sp.*, the precipitation of CaCO<sub>3</sub> involves ion exchange and potential of hydrogen (pH). Bacterial cell walls are negatively charged, thus attract cations from the environment, including Ca<sup>2+</sup>, to be stored on their cell surfaces [7]. Nguyen and Ghorbe reported that the use of *Bacillus sp.* also enhances the compressive strength and reduces the porosity significantly [8].

The application of bacteria as a self-healing agent in concrete requires special treatment, and their growth is difficult to control during the calcination process to repair occurring cracks [7,9]. Yasuhara et al. [10] developed an alternative method, namely enzyme-mediated calcite precipitation (EMCP). This method uses an enzyme directly as a bio-catalyst in the formation of calcite CaCO<sub>3</sub>.

Bacteria can produce enzymes as a bio-catalyst. Bio-catalysts can come from bacteria that contaminate milk. The type of bacteria identified in formula milk

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was *B. subtilis* bacteria, and previous research shows that bacteria can grow at various temperatures for varying lengths of time [11]. The other of *Bacillus sp.*, which contaminate milk was *B. cereus*, *B. subtilis*, and *B. licheniformis* [12]. Hence, it is necessary to evaluate milk's efficacy as an additional material to produce enzymes and its impact on calcite formation of EMCP solution for concrete healing. Furthermore, its effect on the permeability and compressive strength of concrete is examined.

## Materials and Methods

### Materials

The materials used in this study include the following: concrete and the materials for the EMCP solution. Portland composite cement (PCC), sand as fine aggregate, gravel as coarse aggregate, and water were used to prepare the concrete samples. Urea,  $\text{CaCl}_2$ , urease with urease activity of 2950 U/g (020-83242, Kishida Chemical, Osaka, Japan), and the formula milk powder produced by Sarihusada, Yogyakarta, Indonesia, which was applied as the catalyst, were all used in the EMCP solution.

### Methods

#### Material Preparation and Testing

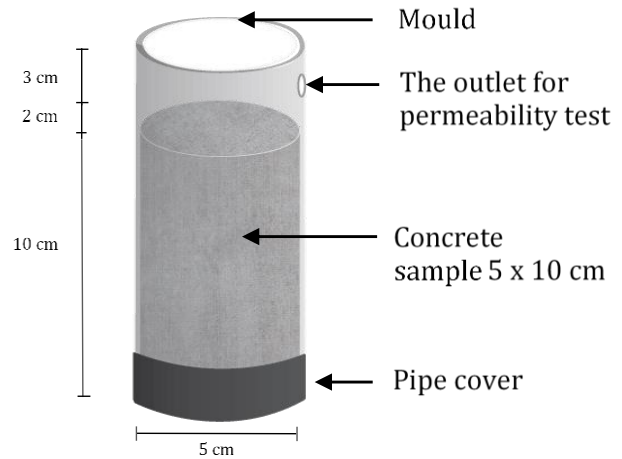
A set of properties tests for aggregates, including specific gravity, water absorption, water content, sludge content, and abrasion, were performed. The summary of the properties of the tested material can be seen in Table 1. The milk used as a catalyst material in the EMCP method in this study was milk in dry powder, which was stored at room temperature before mixing.

**Table 1.**-The Physical Properties of Materials

Parameters	Value	Standard
<b>Fine Aggregate</b>		
Specific gravity (SSD)	2.67	SNI 03-1970-1990 [13]
Absorption (%)	1.21	SNI 03-1970-1990 [13]
Water content (%)	1.52	SNI 03-1971-1990 [14]
Sludge content (%)	2.31	SNI 03-6820-2002 [15]
<b>Coarse Aggregate</b>		
Specific gravity (SSD)	2.53	SNI 03-1970-1990 [13]
Absorption (%)	3.01	SNI 03-1970-1990 [13]
Water content (%)	2.67	SNI 03-1971-1990 [14]
Abrasion (%)	26.11	SNI 2417:2008 [16]

#### Preparation of Concrete

Cylindrical PVC (polyvinyl chloride) moulds were utilized to prepare concrete samples (Figure 1). The proportions of a mixture of coarse aggregate, fine aggregate, cement, and water were calculated based on SNI 03-2834-2000 for 20 MPa compressive strength [17]. The composition of materials for concrete is shown in Table 2.



**Figure 1.** Cylindrical Mould with Concrete Sample

**Table 2.** Concrete Mix Design

Materials	Proportion (Kg)	
	/m <sup>3</sup>	0.0002 m <sup>3</sup>
Cement	418	0.08
Water	205	0.04
Fine Aggregate	565	0.11
Coarse Aggregate	1140	0.22

### The EMCP Solution

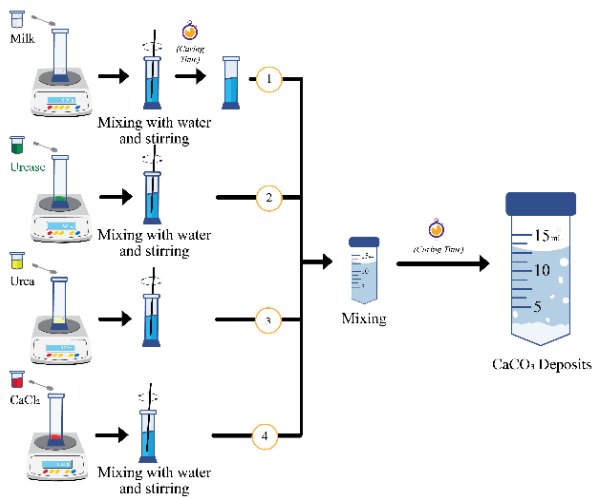
This study adopted a test-tube experiment developed by Putra et al. [18] and Neupane et al [19]. It was carried out to evaluate the calcination process, the amount of precipitated calcite, and the optimum composition of the EMCP solution for  $\text{CaCO}_3$  formation. The EMCP solution consists of urease, urea,  $\text{CaCl}_2$ , and milk. In this study, milk concentrations of 1.25 g/L (e.g., M1-T1, M1-T2, and M1-T3) and 5.00 g/L (e.g., M2-T1, M2-T2, and M2-T3) were added as a catalyst material. Then, the EMCP solution without milk was prepared as the initial EMCP solution (M0-T0). The proportions of the mixture to get the optimum levels of  $\text{CaCO}_3$  are presented in Table 3.

**Table 3.** The Proportions of the Mixture to Get the Optimum Levels of  $\text{CaCO}_3$

Sample	Concentration				
	Urease [g/L]	Urea [mol/L]	$\text{CaCl}_2$ [mol/L]	Milk [g/L] °C	
M0-T0	2.00	1.00	1.00	-	-
M1-T1	2.00	1.00	1.00	1.25	25
M1-T2	2.00	1.00	1.00	1.25	50
M1-T3	2.00	1.00	1.00	1.25	70
M2-T1	2.00	1.00	1.00	5.00	25
M2-T2	2.00	1.00	1.00	5.00	50
M2-T3	2.00	1.00	1.00	5.00	70

The enzymes urease, urea,  $\text{CaCl}_2$ , and milk were prepared separately. Urease, urea, and  $\text{CaCl}_2$  were separately mixed with water and stirred for 5 minutes. Meanwhile, milk was dissolved in water at temperatures of 25, 50, and 70°C. Then the milk

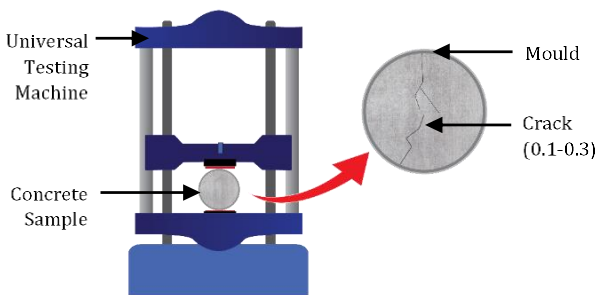
solution was stored for three days at room temperature. Then the milk solution, urease, urea, and  $\text{CaCl}_2$  solution, were mixed in sequence and evenly mixed until all the particles were dissolved. All solutions were stirred evenly and allowed to react in a transparent tube with 12.5 mL volumes for each sample. Subsequently, the sample was cured at room temperature for 3-10 days. After the curing times, the amount of precipitated calcite was evaluated. The grouting solution was sieved through filter paper. Then, particles were held on the filter paper and the tube was dried at  $60^\circ\text{C}$  for 24 hours and thus was calculated as the precipitated mass. The procedures for preparing sample of EMCP solutions in the test-tube experiment is shown in Figure 2.



**Figure 2.** The Procedure for Preparing Sample EMCP Solutions in the Test-tube Experiment

### Curing and Testing Samples

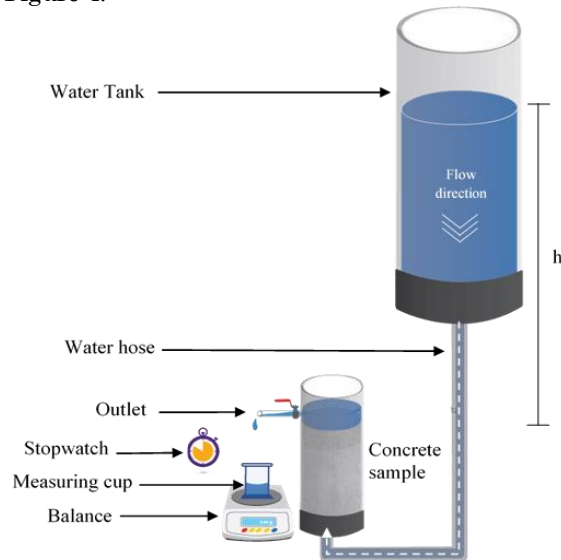
The concrete sample was soaked in a tube during the curing period of 28 days. The confined-splitting test scheme was then applied to the concrete sample to produce a crack with a controlled width of 0.1–0.3 mm. The schematic of the cracking process is illustrated in Figure 3.



**Figure 3.** The Method for Making a Crack in Concrete Samples

The cracked sample was repaired by injecting the selected EMCP solution into the cracked concrete

sample by three circles injection with an interval of three days. Then, permeability and compressive strength tests were carried out. Permeability testing in this study was carried out by following the flow test method, refers to Neupane et al. [20] and the compressive strength of concrete samples was evaluated using universal testing machine refers to SNI-03-1974-2011[21]. Permeability testing procedures is shown in Figure 4.

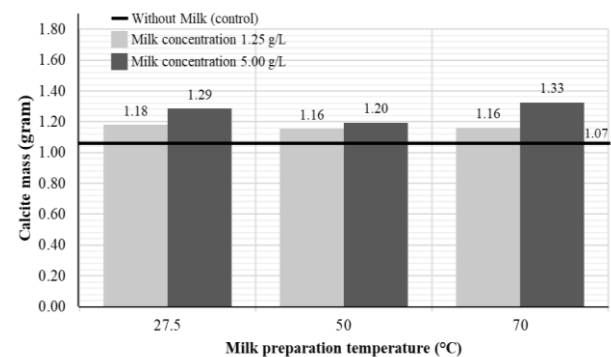


**Figure 4.** The Test Instrument and Permeability Testing Procedure

## Results and Discussion

### Test Tube

Varying the combination of urease, urea  $\text{CaCl}_2$ , and dry milk has been mixed through the precipitation test to obtain the optimum combination. The calcite mass ( $\text{CaCO}_3$ ) formed in the precipitation test of milk concentration is presented in Figure 5.



**Figure 5.** The Results of Calcite Precipitation

The results of precipitation of calcite EMCP solution with the addition of milk tend to produce more calcite mass than the basic EMCP solution. The basic EMCP solution produces a calcite mass of 1.07 gr, while the EMCP solution with the addition of milk produces a

calcite mass between 1.16 and 1.33 gr. The presence of milk may cause optimized urease activity to catalyze urea's hydrolysis and thus promote more calcite mass. The presence of bacteria in the milk may also produce additional enzymes and hence increase urease activity. The bacteria commonly used in the production of bio-concrete are the *Bacillus sp.* and *Sporosarcina sp.* This bacterium is an ureolytic bacterium known to form the urease enzyme [21]. This enzyme acts as a catalyst to convert urea into carbon dioxide and ammonium carbonate, where this enzyme can help calcite sealing [22].

The result also indicates that the preparation temperature has an impact on the precipitation amount. Using the initial temperature of 70°C can improve the amount of calcite by 26%, compared to those of the room temperature. However, the increasing temperature from 27.5°C to 50°C has no significant effect on the calcite mass. Purwanti et al. [23] reported that the preparation's initial temperature has not significantly impacted bacterial growth. In addition, Rowan et al. [24] also reported that about 100 baby milk brands result in similar bacterial growth under varying temperatures.

In this study, milk was contaminated by bacteria. Changes in milk that were contaminated after being stored for three days are shown in Figure 6. The figure shows the colonies are white to yellowish or gloomy white. The edge of the colony is filled but generally uneven. The color characteristics of these colonies are called *Bacillus sp.* [25].

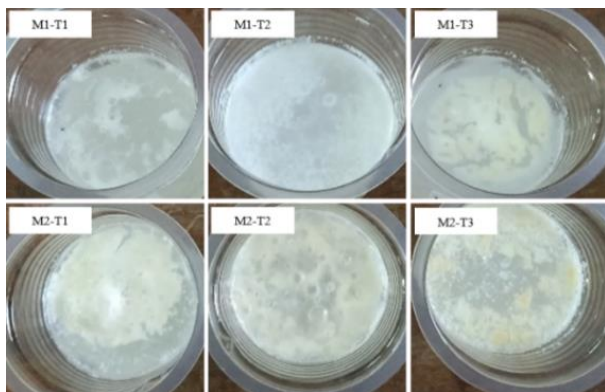


Figure 6. The Changes in Milk after Three Days of Storage

### Permeability and Compressive Strength Test of Concrete

Permeability and compressive strength tests were carried out on specimens before and after repair. Tests were carried out on intact concrete (U), cracked concrete without injection (R), crack repair concrete with control EMCP solution without the addition of milk (I), and crack repair concrete with optimum solution EMCP plus milk (IS). Based on Figure 7, the sample cracks have been covered after repaired with the EMCP solution.

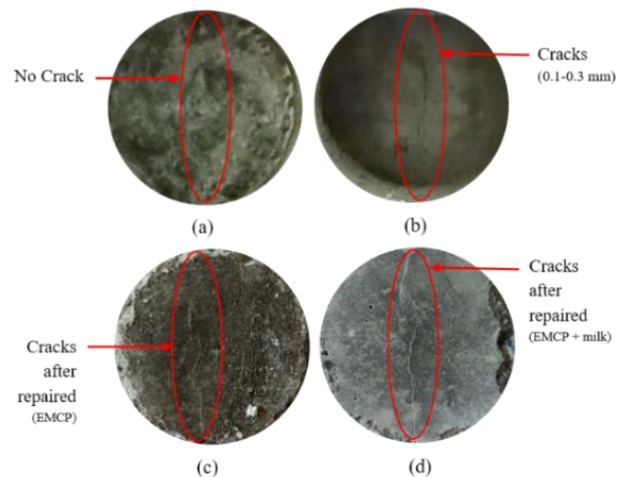


Figure 7. The Cracks in Concrete Samples (a) U, (b) R; (c) I; (d) IS

### Permeability Test of Concrete

Permeability testing in this study was carried out by conducting the flow test method. Concrete permeability is affected by micro-cracks or cracks formed by deformation caused by thermal shrinkage or drying shrinkage and the loading [26]. Changes in the permeability value before and after repair are shown in Figure 8. In addition, Figure 8 also shows the effect of the number of injections on the value of the concrete permeability coefficient.

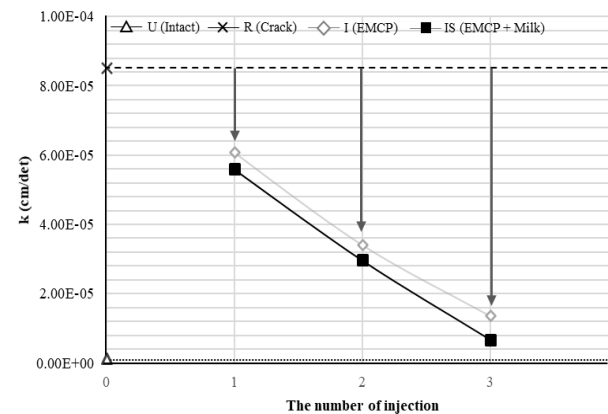


Figure 8. The Relationship of k with the Number of Injections

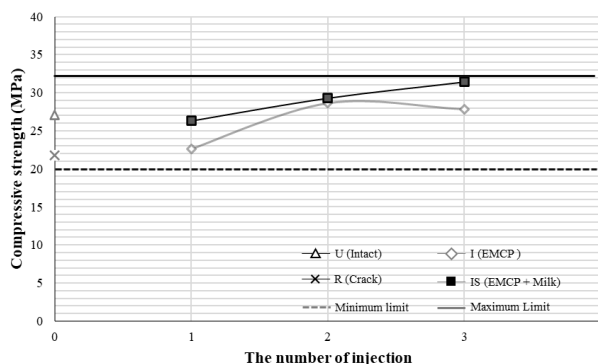
Figure 8 shows that the value of permeability in intact concrete samples (U) is  $1.27 \times 10^{-6}$  cm/s, and the permeability value in cracked concrete without injection (R) is  $8.51 \times 10^{-5}$  cm/s. The results show that cracks in the concrete will affect the permeability coefficient's value, the greater the pore or crack in the concrete, the greater the permeability coefficient [27]. The permeability coefficient value decreases after repairing with the control EMCP solution without the addition of milk (I), and repairing cracks with the optimum EMCP solution with milk (IS). The reduction in the percentage of permeability value

after the first injection was 34.34% for sample (IS) and 28.59% for sample (I) of the permeability value in cracked concrete (R). The decrease in permeability after the second injection was 65.21% for the sample (IS) and 59.95% for the sample (I). Meanwhile, the decrease in permeability value after the third injection was 92.23% for the sample (IS) and 84.09% for the sample (I). The permeability repair of cracked concrete after three rounds of injection is close to the value of the control concrete sample's permeability coefficient without any crack (U).

The decrease in permeability was due to calcite deposits forming and helping to close cracks or gaps in concrete. This result is in line with research reported by Wang et al. [28]. It showed the improvement of cracks in concrete with the Enzyme-Mediated Calcite Precipitation (EMCP) method without and with milk addition could reduce the permeability value. The results also showed that the percentage of permeability reduction in repairing concrete cracks with the optimum solution of EMCP with the addition of milk (IS) tended to be better than repairing concrete cracks with EMCP solution without milk (I). This result shows that adding milk to the EMCP solution increases the effectiveness of the urease enzyme in the formation of calcite deposits [29]. In addition, casein in milk also can bind calcium ions around it, which can help the deposition of calcite [30].

### Compressive Strength of Concrete

Compressive strength testing was carried out on specimens using Universal Testing Machine (UTM). The effect of injection amount on compressive strength is shown in Figure 9.



**Figure 9.** The Relationship of Compressive Strength with the Number of Injections

Based on the graph in Figure 9, the concrete sample's compressive strength value without any crack (U) was 27.06 MPa. This value corresponds to the compressive strength required based on SNI 03-2834-2000. Whereas after the sample was cracked, the compressive strength value decreased from 19.33% to 21.83 MPa. This result showed the influence of cracks

on compressive strength. According to Nakamura et al. [31], crack width affects the decrease in concrete's compressive strength. The reduction in this concrete's compressive strength can reach 80%, accompanied by an increase in crack width in concrete in the plastic zone (before maximum pressure occurs).

The compressive strength value has increased again after being repaired with the control EMCP solution without milk (I) and crack repair with the optimum solution EMCP plus milk (IS) along with the number of injections. The percentage of compressive strength value at the first injection was 20.62% for the sample (IS) and 3.55% for the sample (I) of the compressive strength value in the cracked concrete (R). The percentage of compressive strength at the second injection was 34.27% for the sample (IS) and 31.29% for the sample (I). Meanwhile, the percentage of compressive strength values at the 3<sup>rd</sup> injection was 44.03% for the sample (IS) and 27.62% for the sample (I). The compressive strength value after repairing concrete cracks with three times injections is close to the maximum planned compressive strength limit, specifically for the sample (IS). Based on these data, concrete healing without milk show the repair of cracks in concrete with the Enzyme-Mediated Calcite Precipitation (EMCP) method without and with the addition of milk as a catalyst able to increase the compressive strength of concrete.

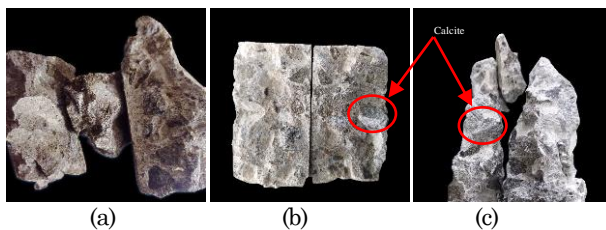
The compressive strength value of samples that had been improved exceeded the compressive strength value of intact concrete (U). The compressive strength value that exceeded this occurs after the second repair injection. The percentage of compressive strength value at the second injection exceeded 8.32% for the sample (IS) and 5.91% for the sample (I) of the compressive strength value in the concrete (U). The percentage of compressive strength values at the 3<sup>rd</sup> injection could exceed 16.19% for the sample (IS) and 2.95% for the sample (I). The highest compressive strength results or improvement of the 3<sup>rd</sup> injection sample (IS) could reach 98.75% of the planned average compressive strength (maximum limit) based on SNI 03-2834-2000. It may occur because the age factor of the concrete still influenced the tested concrete. The compressive strength of concrete increases with age. The age of concrete meant here started since the concrete began to be printed, the rise in the concrete's compressive strength was initially fast, but over time, the rate of increase slowed. So the age for standard concrete compressive strength is 28 days because the concrete does not experience a significant increase in compressive strength after 28 days [32].

In addition, the results showed that the percentage of compressive strength value of repairing concrete cracks with an optimum solution of EMCP - milk (IS)

tended to be higher than repairing concrete cracks with EMCP solution without milk (I). This result showed the possible influence of bacteria present in milk. The bacteria indicated are gram-positive ureolytic bacteria that can produce urease, the *Bacillus sp.* [6]. This urease can catalyze urea's hydrolysis to produce carbon dioxide, ammonia ions, and hydroxides [33]. Carbon dioxide is converted into carbonate ions, which combine with calcium ions of cement hydration products on the surface of the concrete contents to form more calcite deposits than EMCP solutions without the milk. Calcite deposits are used to help close cracks or cracks in concrete. Calcite deposition in this gap will make the compressive strength of concrete to be higher. This result is in line with research conducted by Chen et al. [34].

### Visualization of Calcite Formation in Concrete Samples

The effectiveness of calcite deposition formation for repairing cracks in concrete using the Enzyme-Mediated Calcite Precipitation (EMCP) method with milk addition needs to be known. In this study, calcite deposits formed by adding the EMCP solution to the crack can be seen in Figure 10.



**Figure 10.** The Calcite Deposits in Concrete Samples (a) U and R; (b) I; (c) IS.

Based on Figure 10, the test specimen before the repair was not covered by calcite deposits. Meanwhile, the specimens repaired by EMCP solution without milk (I) or by the addition of milk (IS) were covered with calcite crystals or calcium carbonate [35]. Thus, the repair of cracks in concrete can be done in this study and affects the value of permeability and compressive strength.

### Conclusions

Based on the results obtained, the optimum composition of milk that could be used in the formation of calcite was milk with a 5 gr/l concentration with the initial preparation temperature of 70°C, producing 26% more mass of calcite than the basic EMCP solution. The addition of milk as a catalyst in repairing concrete cracks could affect permeability and compressive strength. After repairing with injection as much as three times, the decrease in the

permeability coefficient could reach 92.23% of the cracked concrete's permeability coefficient value. In comparison, the compressive strength value reaches 98.75% of the compressive strength of the planned average compressive strength.

### Acknowledgment

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