

## Influence of Different Concentration of Tris Buffer Solution on Calcium Carbonate Precipitation in Bio-based Repair Materials

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**Abstract**— This research examined differences in the concentration of Tris buffer solution on calcium carbonate precipitation in bio-based repair materials. Four concentrations of Tris buffer solution were chosen based on previous research with initial pH 8.0 and 9.0. Initial experiments demonstrated that the concentration and pH of Tris buffer solution had an influence on the amount of precipitation of calcium carbonate. X-Ray Diffraction (XRD) analysis elucidated the morphological and structural differences of the calcium carbonate crystal, including calcite and vaterite. They are the prominent forms of CaCO<sub>3</sub> detected based on results obtained by according to FT-IR analysis. The result further explains the effectiveness of Tris buffer concentration.

**Keywords**—Bio-based repair materials; tris buffer solution; calcium carbonate precipitation; fourier-transformed infra-red spectroscopy (FT-IR); X-Ray rirfraction (XRD).

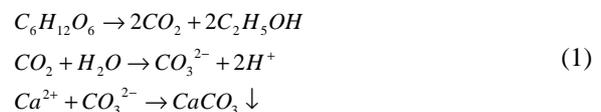
### I. INTRODUCTION

Recently, liquid-based repair materials in the field of self-healing through the use of microbially induced precipitation (MIP) have been investigated (1–9). Kawasaki et al. (1) tried to apply such repair materials using biotechnology to ground improvement. The bio-based repair material contains yeast, organic nutrient source and calcium source. Carbon dioxide produced by microbial metabolism according to Eq. (1) provides carbonate ions leading to calcium carbonate precipitations formed by calcium source mixed in bio-based repair materials. In this study, it was shown that crystals of calcium carbonate are precipitated by bio-based repair materials utilizing yeast microbial metabolism. Ujike et.al (5) and Kawaai et al. (6) has investigated some considerations on precipitation rate of calcium carbonate in bio-based materials. Putri et al. (7,8) does also examined the effects of temperature and the types of dry yeast on precipitation rate of calcium carbonate. It was shown all the types of dry yeast commercially which was tested on the research can be used as a basic constituents in the bio-based repair materials.

Calcium carbonate, as one of the most widely existing bio-minerals produced by organism, has three different crystal polymorph, such as calcite, aragonite and vaterite. The phase diagram of CaCO<sub>3</sub> shows that calcite is the stable style under ambient conditions, aragonite is the high-

pressure polymorph, and vaterite is thermodynamically unstable (10). From the viewpoint of thermodynamics, calcite is more stable than aragonite. Vaterite is the least stable of these three phases and easily transforms into calcite in a chemistry laboratory. Although the amount of vaterite is far less than that of calcite and aragonite, it also plays key roles in biological life and health (11).

In the bio-based repair materials, the CaCO<sub>3</sub> precipitates according to the following reactions:



The material produced through the reactions does not adversely affects the concrete materials because the precipitates are mainly tied of calcium carbonate which is one of the reaction products formed by carbonation of hydration products. Therefore, it is advantageous if the reaction products have similar materials properties of concrete.

In addition to the material properties, the mixture is a less viscous material in contrast to conventional repair materials. This may overcome shortcomings associated with the traditional repair materials. Thus, it would be useful if the mixtures infiltrate into deeper zones of gaps formed between

concrete members and could effectively improve the water tightness of concrete along with the defects.

Conventionally, injecting resin or cementitious materials for repairing gaps and cracks in concrete structures has been used (12,13). Resin-based materials such as epoxy resin are resistant to acid/alkali and do not cause corrosion, and have advantages such as small elastic coefficient and excellent adhesion. However, there are drawbacks such as adverse effects on the repairing effect due to water contamination and adverse effects on adhesion when the concrete surface is wet, and there is also concern about ultraviolet deterioration (14). Therefore, it seems thought that it is not suitable for repairing water leakage part where defect part is not specified. Based on the above background, this study investigated the development of new repair materials and the applicability to actual structures.

Refers to the previous research, the precipitation rate, particularly at early stages is depending on the concentrations and initial pH levels (6). Correspondingly, on this research, the influences of the concentrations of Tris buffer solution was examined to increase the precipitation rate in bio-based repair materials comprising yeast, glucose and calcium acetate up to 72 hours of elapsed time. The precipitates were also tested for Fourier-Transformed Infra-Red spectroscopy (FT-IR) and X-Ray Diffraction (XRD) analysis for mineral identification formed through the microbial metabolic process of bio-based materials in mixtures.

## II. MATERIAL AND METHOD

### A. Selection of Basic Constituents of Mixtures

Alongside this research, calcium carbonate precipitation was energized by the microbial metabolic process of bio-materials mixtures as shown in Fig. 1. In addition to the reaction, it is important to manage the pH levels in mixture to encourage the precipitation of calcium carbonate. Tris (Tris-hydroxymethyl aminomethane) buffer solution with an alkali buffering capacity was utilized in this research. The initial pH of the Tris buffer solution was adjusted to 8.0 or 9.0 utilizing hydrochloric acid. The utilization of hydrochloric acid ought to be limited as small as possible in adjusting pH levels for guaranteeing no adverse impact on the hardened concrete integrity.

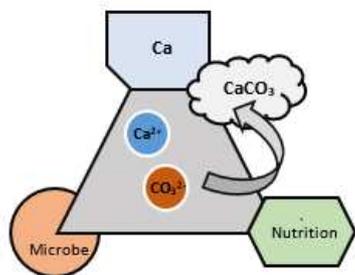


Fig. 1 Basic constituents of bio-based repair material

The provenance of calcium ions utilized for mixtures was selected by examining over higher solubility and no negative impact on concrete materials. The material should also be commercially available and based on cost-effective

assumption. Calcium acetate was chosen in this research based on that consideration.

Besides, dry yeast commercially available was utilized as a microorganism is an anaerobic microorganism and active in the oxygen-free environment. The organic carbon sources are necessary for the metabolism of the microorganism. Through the anaerobic fermentation, the yeast converts carbohydrates to carbon dioxide and alcohols in the form of glucose ( $C_6H_{12}O_6$ ) which was selected as an organic carbon source.

It is realized that highly alkaline environment which pore solution in concrete shows is a severe condition for the yeast. It is essential for the microorganism to exist in the mixtures which have relatively lower pH, which is initially adjusted as low as possible. As indicated by previous research, it has been examined that the calcium carbonate precipitation was likely to be less when pH decreased less than 7.5 (5).

In order to maintain the appropriate pH levels in mixtures tested, the pH should initially adjust as 8.0 or 9.0 in room conditions controlled at 20°C.

### B. Selection concentration of tris buffer solution

The composition of liquid medium for each group is shown in Table 1, where letters A and B in group label represent initial pH of liquid, 8.0 and 9.0 respectively. For each group, the concentration of Tris buffer solution was adjusted to 0.25 mol/L, 0.5 mol/L, 0.75 mol/L and 1.0 mol/L, respectively. For each concentration of Tris solution, the mix proportion was set as basic composition (series 1), twofold (series 2), threefold of basic composition (series 3) and fourfold of basic composition (series 4), respectively. The mixtures were tested in dry temperature conditions controlled at 20°C up to 72 hours of elapsed time.

TABLE I  
MIX PROPORTION OF DRY YEAST, GLUCOSE, CALCIUM ACETATE FOR EACH VARIATION

Cases	Dry yeast (g/L)	Glucose (mol/L)	Calcium acetate (mol/L)	Tris buffer concentration (mol/L)
A1	9.0	0.1	0.05	
A2	18.0	0.2	0.10	0.25; 0.5;
A3	27.0	0.3	0.15	0.75; 1.0
A4	36.0	0.4	0.20	
B1	9.0	0.1	0.05	
B2	18.0	0.2	0.10	0.25; 0.5;
B3	27.0	0.3	0.15	0.75; 1.0
B4	36.0	0.4	0.20	

### C. Testing Procedures

In order to measure the concentration of calcium ion and the pH in mixtures after the reaction was started, each mixture was prepared in a test tube. First, each material based on mix proportion specified as shown in Table 1 was prepared, and they were mixed with Tris buffer solution in a beaker. The mixture in the glass was subsequently stirred until each material was dissolved into the solution. The Tris buffer solution was further added to make solution 60 mL totally. The test tubes were placed in conditions controlled at 20°C for the measurement. The concentrations of calcium ions and pH were measured in mixtures using commercially available meters (pH/mv meter model SK-620PH and calcium ion electrode model CA-2031). Each test was

carried out using two test tubes to confirm the consistency of results obtained.

Based on the results of calcium ions measured, decreasing rate of calcium ions and precipitated calcium carbonate were estimated using the following formula:

$$\text{Decreasing rate of calcium ions} = \frac{C_0 - C_a}{C_0} \quad (2)$$

$$\text{CaCO}_3 = Q \left( \frac{\text{mol}}{\text{L}} \right) \times m (\text{L}) \times M \left( \frac{\text{g}}{\text{mol}} \right) \times \frac{C_0 - C_a}{C_0} \quad (3)$$

Where  $\text{CaCO}_3$ : the amount of precipitates (g), Q: concentration of calcium acetate (mol/L), m: the amount of solution (L), M: molar mass of calcium carbonate (100.09),  $C_0$ : Initial concentration of calcium ion and  $C_a$ : concentration of calcium ion measured.

The precipitates were also tested for Fourier-Transformed Infra-Red spectroscopy (FT-IR) and X-Ray Diffraction (XRD) analysis for mineral identification formed through the microbial metabolic process of bio-based materials in mixtures.

#### D. Characterization of calcite precipitation by FT-IR and XRD

The calcium carbonate precipitation produced in test tubes were analyzed using Fourier-Transformed Infra-Red spectroscopy (FT-IR). Infrared spectroscopy is a technique based on the vibrations of the atoms of a molecule. It is used for the determination of molecular structure or the identification of chemical species. A vibration will be infrared active if a change in the dipole moment of the molecule occurs during the vibration. An infrared spectrum consists of a series of bands or peaks which result from the absorption by a part of the molecule of the incident radiation at a particular energy (15). FT-IR spectroscopy is especially favoured due to its versatility and non-destructiveness. It is a fast and cost-effective technique, as it does not require a sample preparation step (16).

FT-IR spectra were collected on a Thermo Scientific Nicolet iS5 FT-IR Spectrometer series spectrometer equipped with Universal Attenuated Total Reflexion (ATR) unit. The spectra were recorded in the range of 7800–350  $\text{cm}^{-1}$  optimized, mid-infrared KBr beamsplitter with sensitivity 8000:1 peak to peak in five seconds, 22000:1 peak to peak in one minute (17). The ATR analysis requires a tiny amount of sample (<5 mg), and furthermore, no preparation or dilution of the sample is needed. The FT-IR was first calibrated for background signal scanning, and then the experimental sample scanning was conducted. The spectra were normalized in order to compare them.

The precipitation also analyzed using X-ray Diffraction (XRD). XRD analysis was carried out using the residue left in the test tube after 24 hours, 48 hours and 72 hours. Each sample was crushed and pulverized to an average particle size of less than 10 microns, and then mounted onto an instrument-specific plastic slide plates. Then the mass absorption coefficient of the sample was determined by X-ray transmission. The XRD pattern was obtained by scanning from 5 to 50 degrees, 2 thetas using a vertical X-ray diffractometer. The components of the sample were identified by comparing them with database powder diffraction PDF-4/Mineral 2015 established by the

International Center for Diffraction Data (18). XRD is a good evaluation technique to assess the functionality of the bacteria-based repair system and to evidence the involvement of bacteria in the mineral production.

### III. RESULTS AND DISCUSSION

#### A. Influence of different concentration of Tris Buffer Solution on calcium carbonate precipitation

The effect of different concentration of Tris buffer solution was examined on the precipitation rate up to 72 hours later after mixed. Decreasing rate of calcium ion was calculated based on Eq. (2). The decreasing rate of calcium ion is directly related to the amount of calcium carbonate precipitation as shown in Eq. (3). According to the previous research (7–9), it has been reported that the decreasing rate of calcium ion has a good correlation with the precipitation rate of calcium carbonate by measuring filtered paper. The decreasing rate of calcium ion was found almost to be equal to precipitation of calcium carbonate. Therefore, the higher decreasing rate of calcium ions indicates that the precipitation rate of calcium carbonate is higher. In this paper, decreasing rate of calcium ion is used as an index of precipitation rate of calcium carbonate. Furthermore, calcium ions present in the mixtures were mostly converted into calcium carbonate which precipitated in the mixtures (6).

The mixtures tested were the A1 cases as shown in Table 1. Fig. 2 shows the decreasing rate of calcium ions and changes of pH measured in the mixtures tested.

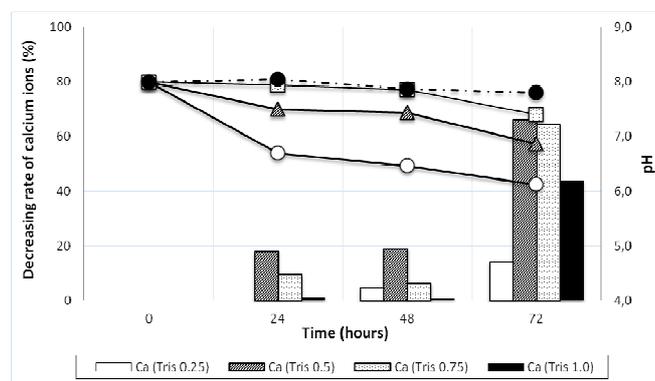


Fig. 2 Concentration of Calcium Ions and pH (case A1, initial pH 8.0)

As can be seen, the concentration of calcium ions in the A1 mixture for the concentration of Tris buffer 0.25 mol/L was not decreased after 24 hours. And then, the decreasing rate reached about 5% after 48 hours elapsed time. The decreasing rate of calcium ions reached about 14% after 72 hours. On the other hand, the decrease in the concentration of calcium ion in the A1 mixture for the concentration of Tris buffer 0.5 mol/L were notably observed after 24 hours of elapsed time. In this case, the decreasing rate of calcium ions is higher than those of concentration 0.25 mol/L, 0.75 mol/L, and 1.0 mol/L. On the concentration of Tris buffer solution adjusted to 0.5 mol/L, the decreasing rate reached about 18% after 24 hours, the concentration of calcium ions slightly decreased to 19% after 48 hours. And then, the decreasing rate reached about 66% after 72 hours of elapsed time. For the concentration of Tris buffer 0.75 mol/L and 1.0

mol/L, the decreasing rates of calcium ions showed below 10% up to 48 hours of elapsed time. After 72 hours, the concentrations of calcium ions was reduced to 65% and 44% for Tris buffer solution with 0.75 mol/L and 1.0 mol/L respectively.

Otherwise, for the concentration of Tris buffer solution with 0.5 mol/L, the pH decreased from 8.00 to 7.5 after 24 hours. It was slightly decreased to 7.44 after 48 hours and reached about 6.87 after 72 hours of elapsed time. In the same way, for the concentration of Tris buffer 0.25 mol/L, the pH significantly decreased from 8.0 to 6.13 after 72 hours of elapsed time. For the concentration of Tris buffer solution with 0.75 mol/L, the pH decreased to 7.94 after 24 hours of elapsed time. It reached 7.86 after 48 hours and reduced to 7.40 after 72 hours of elapsed time. Moreover, for the concentration of Tris buffer solution with 1.0 mol/L, the pH slightly decreased about 7.80 after 72 hours of elapsed time.

Fig. 2 was shown small of calcium carbonate precipitation amount at the early stage. The precipitation reached more than 20% after 48 hours. Under this experimental condition, the basic composition (A1 mixture) mixed in Tris buffer solution seemed to be adequate in the case of concentrations between 0.5 mol/L to 0.75 mol/L, which is illustrated by the higher decreasing rate observed after 72 hours of elapsed time.

In order to determine appropriate concentrations of Tris buffer solution depending on the initial pH, A2 to B4 mixtures were tested based on the results of the A1 mixture. They were comprised of higher concentrations of the yeast mixed in four series of Tris buffer solution with initial pH of 8.0 or 9.0. Fig. 3 shows that the decreasing rate of calcium ions measured after 24 hours of elapsed time in each Tris buffer solution.

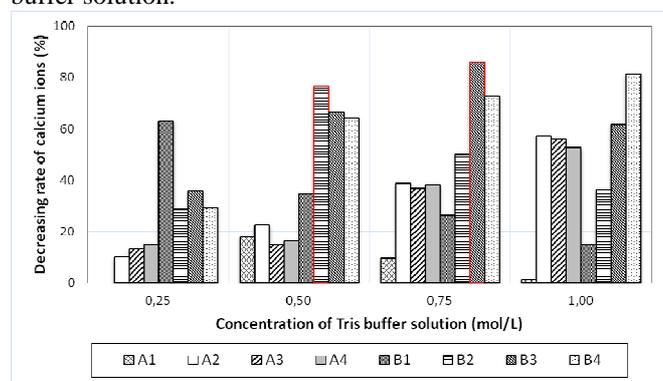


Fig. 3 Decreasing rate of calcium ions after 24 hours

In the case of B1 mixture mixed in Tris buffer solution with an initial pH of 9.0, the precipitation seemed to be higher in the cases of the solution adjusted to a concentration of 0.25 mol/L, which reached greater than 60% of the decreasing rate after 24 hours. The pH measured in each mixture after 24 hours was 8.43, 6.16, 6.04, and 6.03 for B1 to B4 mixtures respectively.

The result suggested that the use of a low concentration of basic constituent of mixture led to the faster generation of carbonate ions, which is beneficial for the precipitation of calcium carbonate.

On the other hand, in the cases of the solution adjusted to the concentration of 0.5 mol/L, the decreasing rate of

calcium ions seemed to be higher in the case of B2, B3, and B4 mixtures. The decreasing rate of calcium ions which reached greater than 60% for B2, B3, and B4 mixtures. The pH measured in each mixture after 24 hours was 8.09, 6.30 and 6.14 for B2, B3, and B4 mixtures respectively. This precipitation process has similar tendency to decreasing rate of calcium ions which observed in the B3 and B4 mixtures mixed in Tris buffer solution adjusted with 0.75 mol/L and 1.0 mol/L respectively. On these conditions, the results suggested that the use of a higher concentration of basic constituents mixtures led to the higher amount of calcium carbonate precipitation.

Besides that, in the case of A2 to A4 mixtures mixed in Tris buffer solution with the initial of pH 8.0, the precipitation seemed to be adequate in the case of the solution adjusted to the concentration of 1.0 mol/L, which reached greater than 53% of the decreasing rate at the time. The pH measured in each mixture after 24 hours was 7.38, 6.08 and 6.00 for A2, A3, and A4 respectively. In the same concentration of Tris buffer solution (1.0 mol/L), the cases of B3 and B4 mixtures also has the higher decreasing rate of precipitation. It reached greater than 60% of the decreasing rate after 24 hours of elapsed time. The pH measured in B3 mixture which showed the highest decreasing rate of calcium ions tested was 6.04 for Tris 0.25 mol/L, 6.3 for Tris 0.5, 7.79 for 0.75 mol/L and 8.48 for 1.0 mol/L. Thus, pH levels in the region of alkali environment were maintained for the latter case. The highest decreasing rate can be explained by the fact that the precipitation process could be prolonged owing to the use of the solution with higher alkali buffering effect.

Based on the results above, recommended that the concentration of Tris buffer solution should have been adjusted depending on the initial pH for facilitating the precipitation processes. Therefore, the utilization of a lower pH i.e. 8.0 required a relatively higher concentration of Tris buffer solution especially for the cases of mixtures comprising a larger amount of yeast.

Fig. 4 and 5 show the results of the decreasing rate of calcium ions and pH up to 72 hours of elapsed time for all series. Based on Fig. 4, two groups are confirmed. The mixture with the lowest concentration of Tris buffer solution (0.25 mol/L) led the significant changes of pH in the mixture after 24 hours. The pH kept lower than 6.5, and the decreasing rate of calcium ion reached lower than 20%. Similarly, pH for the mixtures with the concentration of Tris buffer solution 0.5 mol/L and 0.75 mol/L also reached below 6.5. Except, for A1 mixture, it reached about pH 7.5 and pH 7.0 for the concentration of Tris buffer with 0.5 mol/L and 0.75 mol/L respectively.

On the other hand, the higher concentration of mixtures tested and mixed in a higher concentration of Tris buffer solution (1.0 mol/L) also led to lower pH (shown by the arrow in Fig. 4). The pH decreases to 6.5 or less. But, the decreasing rate of calcium ion reached the higher amount. It reached about 60% of decreasing rate of calcium ion. With the exception of A1 mixture, the pH kept about 7.5. It showed a low concentration of mixture led to higher pH. This is because the yeast's metabolism is not active and carbon dioxide generation is small.

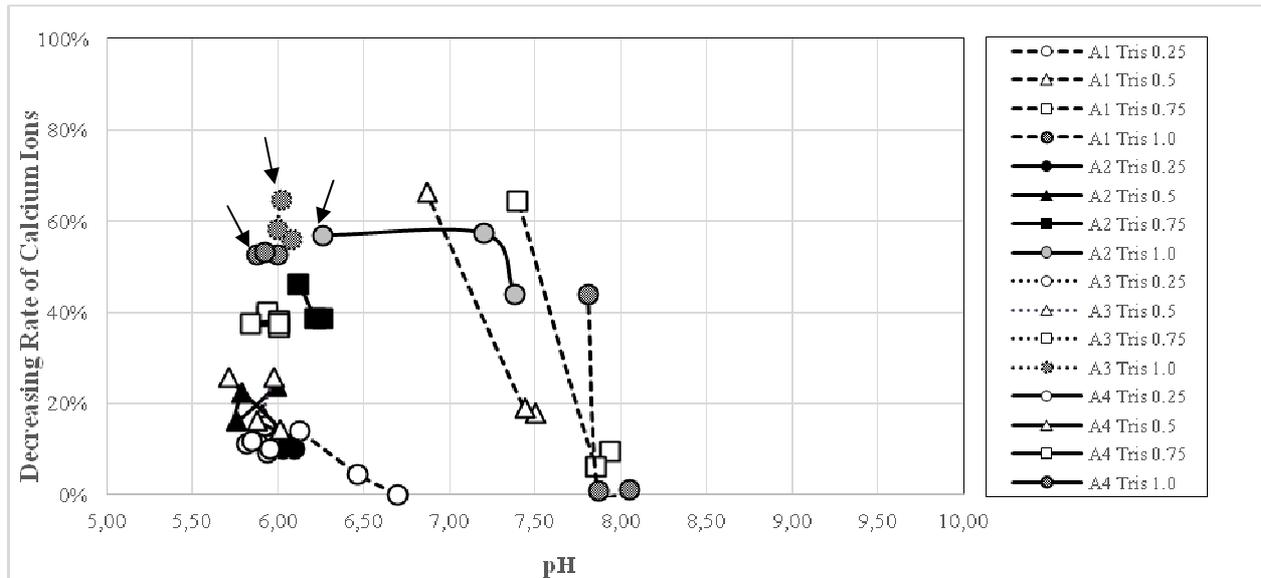


Fig. 4 Decreasing rate of calcium ions and pH (initial pH 8.0)

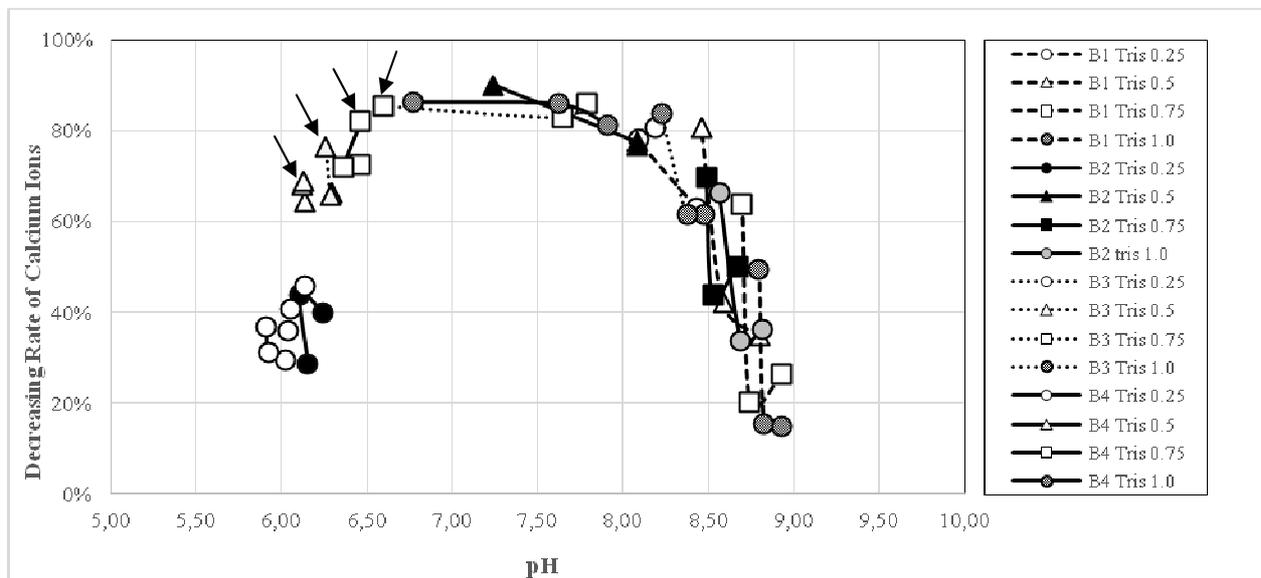


Fig. 5 Decreasing rate of calcium ions and pH (initial pH 9.0)

Otherwise, Fig. 5 show the decreasing rate of calcium ions and pH for initial pH adjusted to 9.0. As can be seen, the mixture with the highest concentration of Tris buffer solution i.e. 1.0 mol/L in each mixture, the changes of pH in the mixtures after 24 hours were marginal, except the mixture with high concentration of yeast (B4 mixture). For the other concentration of Tris buffer (0.5 mol/L and 0.75 mol/L) the pH drop rapid after 24 hours for B3 and B4 mixtures. It reached lower than 6.5 (shown by the arrow in Fig. 5). The maximum precipitation amount of calcium carbonate reached on B2 mixture when adjusted in 0.5 mol/L to 0.75 mol/L.

In this study, the concentration of Tris buffer solution was found to be adequate in the ranges of 0.5 to 0.75 (mol/L) in the case of an initial pH adjusted to 9.0 and 0.75 to 1.0 (mol/L) in the case of an initial pH adjusted to 8.0. This

result was confirmed by the previous research [4]. It was found that the generation of carbonate ions was faster in the mixture with relatively pH 9.0 adjusted initially.

#### B. Fourier-Transformed Infra-Red Spectroscopy (FT-IR) and X-Ray Diffraction (XRD) Analysis

It is important to find the best compositions contributing to the larger amount of precipitates as crystals as well as faster reaction rate especially at early stages, which could be more effective in sealing the leakage parts in concrete. FT-IR and XRD were conducted with respect to the highest precipitation on the tube testing, i.e. B2 mixture for Tris buffer solution 0.5 mol/L and B3 mixture on Tris buffer solution 0.75 mol/L.

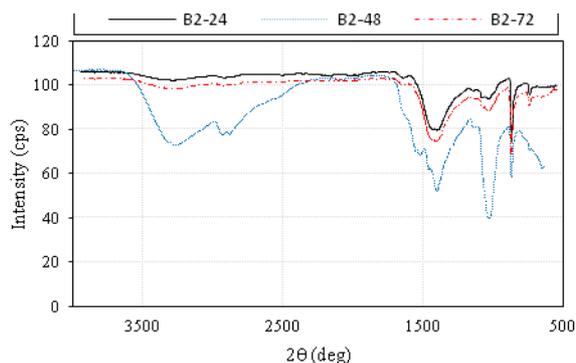


Fig. 6 FT-IR Spectra for precipitate formed on B2 mixture, tris buffer 0.5 mol/L

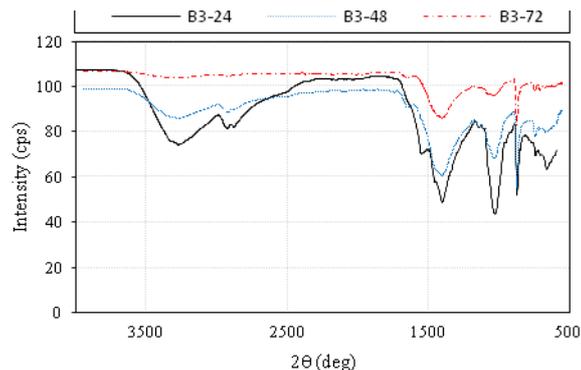


Fig. 7 FT-IR Spectra for precipitate formed on B3 mixture, tris buffer 0.75 mol/L

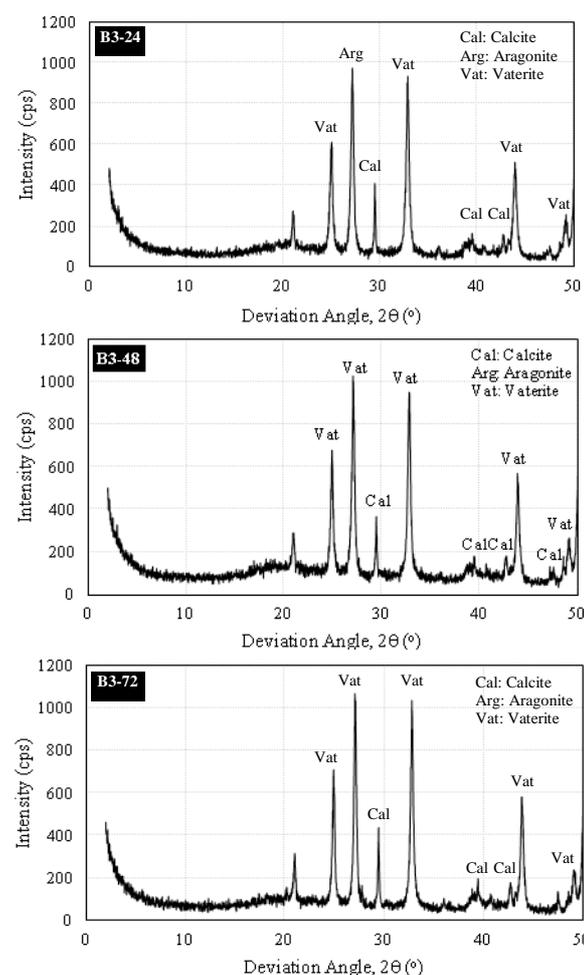
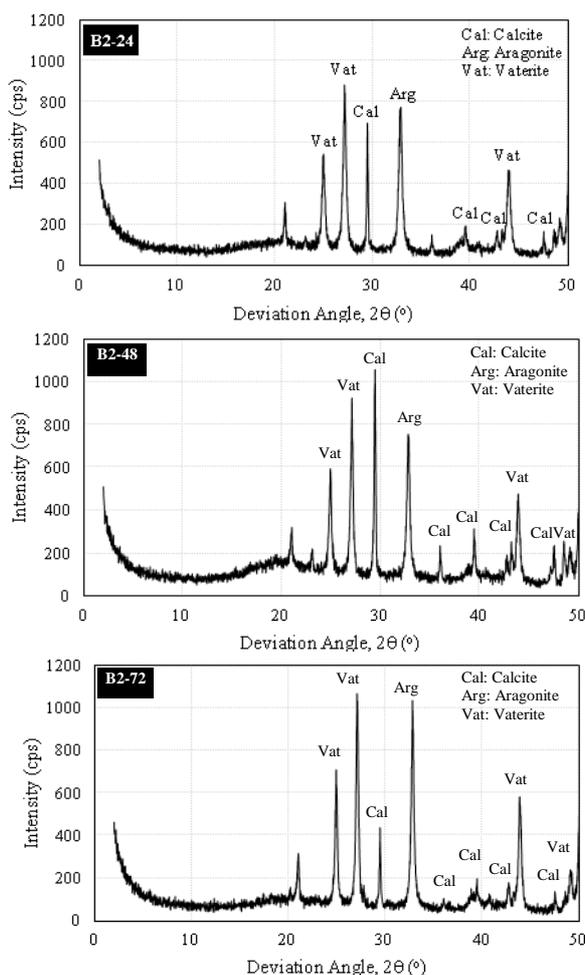


Fig. 8 XRD Pattern obtained at B2 and B3 mixtures

FT-IR spectroscopy was used to identify the chemical selection of the precipitate formed in bio-based materials (3). The IR Spectra were recorded using Thermo Scientific Nicolet iS5 FT-IR Spectrometer and stores using a spectroscopic software (Omnice software)(17).

The occurrence of the major transmittance bands specific to calcium carbonate was observed. Fig. 6 shows FT-IR spectra for the precipitate produced by the B2 mixture in the concentration of Tris buffer solution of 0.5 mol/L. After 24 hours of elapsed time, the peaks at 1403.92, 995.089 and 873.596 indicated the presence of calcium carbonate. For

precipitation up to 48 hours elapsed time, the signals at 1403.92, 1031.73 and 871.67 show the presence of calcium carbonate respectively. Moreover, precipitation after 72 hours also indicates the presence of  $\text{CaCO}_3$ , the peaks at 1403.92, 1035.59, and 871.67. These spectra were compared to the standard spectrum of calcium carbonate from NIST Standard Reference Database (19).

Correspondingly, a similar tendency was observed as shown in Fig. 7. It shows spectra in precipitation of the B3 mixtures mixed in Tris buffer solution of 0.75 mol/L. The result was compared to the standard spectrum of calcium

carbonate, which was in good agreement with the peaks 1403.92, 1029.80, 871.67 (after 24 hours); 1398.14, 1029.80 and 871.67 (after 48 hours); and also 1398.14, 1029.80, and 871.67 (after 72 hours elapsed time).

This result shows the presence of calcium carbonate on the bio-based materials, thus showing that dry yeast mediated the CaCO<sub>3</sub> precipitation. The FT-IR spectra of the B2 and B3 mixtures are indicative of the presence of calcium carbonate on the precipitation formed of bio-based materials. The similar precipitation assessed by XRD analysis to confirm the polymorph of crystals.

For the B2 mixture, the XRD patterns of the precipitates obtained in the presence of CaCO<sub>3</sub> are presented in Fig. 8. In the precipitation after 24 hours, the three polymorph of CaCO<sub>3</sub> (aragonite, calcite, and vaterite) coexisted. Vaterite and calcite were the dominant polymorphs. There were 4 peaks of vaterite and calcite respectively. However, after 48 hours, intensities on calcite increased. Correspondingly, after 72 hours calcite was the dominant polymorph of CaCO<sub>3</sub> material as shown in 5 peaks of calcite in XRD pattern. Similarly, in the B3 mixtures, there were only calcite and vaterite polymorph in the calcium carbonate precipitation. After 24 hours of elapsed time, there were 5 peaks of vaterite and 3 peaks of calcite. There was no peaks indicating the presence of aragonite in this pattern. After 48 to 72 hours of elapsed time, showed the same pattern. Vaterite is the dominant polymorph of CaCO<sub>3</sub>. Accordingly, a high concentration of tris buffer solution promotes the vaterite phase in experimental temperature.

#### IV. CONCLUSIONS

The result showed that calcium carbonate precipitates depending on the different concentration of tris buffer solution within 72 hours after mixing. In this study, the concentrations of Tris buffer solution were found to be adequate in the range of 0.5 to 0.75 (mol/L) in the case of an initial pH 9.0 and 0.75 to 1.0 (mol/L) in the case of an initial pH of 8.0. In the case of mixtures mixed in initial pH of 9.0, the rate of initial reaction tended to be faster, which could be attributed to the lower concentration of Tris buffer solution adjusted to a relatively higher pH in the mixture.

Based on the precipitation results were apparently assessed by Fourier-Transformed Infra-Red Spectroscopy (FT-IR) analysis, confirmed dry yeast mediated the CaCO<sub>3</sub> precipitation. Also, the FT-IR spectra for precipitation in the test tube are indicative of the presence of calcium carbonate. Furthermore, the XRD analysis confirmed vaterite and calcite are dominant polymorphs in the calcium carbonate precipitation. Also, the results showed that a high concentration of Tris buffer solution promotes the vaterite phase in the calcium carbonate precipitation.

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